09/700751

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 28 May 2001 (28.05.01)	
International application No. PCT/IL00/00550	**Applicant's or agent's file reference** 125955.5 DAB
International filing date (day/month/year) 08 September 2000 (08.09.00)	**Priority date** (day/month/year) 10 September 1999 (10.09.99)
Applicant FISHMAN, Pnina	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   29 March 2001 (29.03.01)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Claudio Borton
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

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NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

REINHOLD COHN AND PARTNERS
P.O. Box 4060
61040 Tel Aviv
ISRAËL

Date of mailing (day/month/year) 08 March 2002 (08.03.02)	
Applicant's or agent's file reference 125955.5 DAB	**IMPORTANT NOTIFICATION**
International application No. PCT/IL00/00550	**International filing date** (day/month/year) 08 September 2000 (08.09.00)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
CAN-FITE TECHNOLOGIES LTD. Achad Ha'am Street 21 61040 Tel Aviv Israel	IL	IL
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [X] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
CAN-FITE BIOPHARMA LTD. 10 Bareket Street Petach Tikva 49170 Israel	IL	IL
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Jean-Marie MCADAMS
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

09/700751

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
125955.5 DAB		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL00/00550	08/09/2000	10/09/1999
International Patent Classification (IPC) or national classification and IPC A61K31/00		
Applicant CAN-FITE BIOPHARMA LTD. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
29/03/2001	25.07.2002
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Beeck, M Telephone No. +49 89 2399 8473

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IL00/00550

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-41 as originally filed

Claims, No.:

1-35 as received on 10/04/2002 with letter of 10/04/2002

Drawings, sheets:

1/15-15/15 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2-9,11-13,20,22,31,35
	No:	Claims	1,10,14-19,21,23-30,32-34
Inventive step (IS)	Yes:	Claims	2,4-9,11,13,20,22,31,35
	No:	Claims	1,3,10,12,14-19,20,22,31,35

Industrial applicability (IA)	Yes:	Claims	1-35
	No:	Claims	

2. Citations and explanations
see separate sheet

SECTION IV:

The separate inventions are:

a) Use of A3RAg etc for inducing G-CSF secretion (Claims 1 to 9)

b) Use of A3RAg etc for inducing proliferation or differentiation of bone marrow or white blood cells (Claims 10 to 13)

c) Use of A3RAg etc for the prevention or treatment of leucopenia (Claims 14 and 15)

d) Use of A3RAg etc for the prevention or treatment of toxic side effects of a drug (Claims 16 to 25)

e) Use of A3RAg etc for the inhibition of abnormal cell growth (Claims 26 to 35)

They are not so linked as to form a single general inventive concept (Rule 13.1 PCT) because the different uses a) to e) are not interrelated.

SECTION V:

1) The opinion expressed as to novelty, inventive step and industrial applicability only refers to matter for which an international search report has been drawn up.

2) The documents are numbered according to their sequence in the search report.

3) The examination has been carried out assuming that the priority is valid.

4) Document D4 already discloses the use of adenosine A3 receptor agonists as antitumour agents (see page 3, line 24, to page 5, line 21, and page 15, line 6, and claim 14).

Therefore the subject-matter of claims 26 to 29, 32 and 34 is not novel (Article 33 (2) PCT).

Document D14 describes that adenosine stimulates the proliferation of bone marrow cells and G-CSF production.

Therefore the subject-matter of claims 1 and 10 is not novel.

Document D19 describes the effect of adenosine and its derivatives for the treatment of leucopenia and cancer (see the claims). It also describes the combination with anti-cancer drugs and the prevention of drug-induced leucopenia.

This document anticipates the subject-matter of claims 14 to 19, 21, 23 to 27, 29, 30, 32, 33 and 34.

5) The subject-matter of claims 3 and 12 differs from what is disclosed in document D14 in that the composition is for oral administration. Since this is an obvious alternative for the administration of pharmaceuticals, the subject-matter of claims 3 and 12 does not involve an inventive step.

6) The subject-matter of claims 2, 4 to 9, 11, 13, 20, 22, 31 and 35 is novel and involves an inventive step.

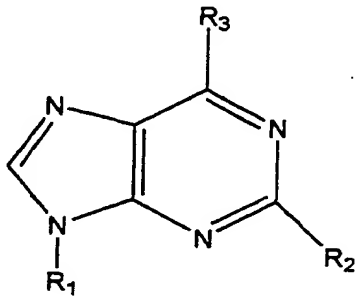
NEW CLAIMS:

1. Use of an effective amount of an active ingredient for the preparation of a pharmaceutical composition for achieving a therapeutic effect, the therapeutic effect comprising inducing G-CSF secretion or production, wherein said active ingredient is selected from an adenosine A3 receptor agonist (A3RAg), an A1 adenosine receptor agonist (A1RAg) or a combination of an A3RAg and an A1RAg.

2. Use according to Claim 1, wherein said active ingredient is A3RAg.

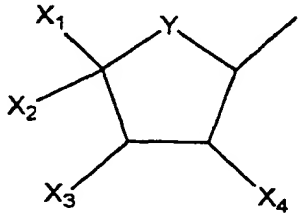
3. Use according to Claim 2, for the preparation of a pharmaceutical composition for oral administration.

4. Use according to Claim 1, wherein said active ingredient is a nucleotide derivative of the following general formula (I):



(I)

wherein R_1 is C_1-C_{10} alkyl, C_1-C_{10} hydroxyalkyl, C_1-C_{10} carboxyalkyl or C_1-C_{10} cyanoalkyl or a group of the following general formula (II):



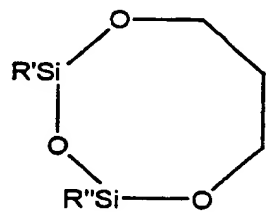
(II)

in which:

- Y is oxygen, sulfur of carbon atoms;
- X_1 is H, C_1-C_{10} alkyl, $R^aR^bNC(=O)$- or HOR^c-, wherein R^a and R^b may be the same or different and are selected from the group consisting of

hydrogen, C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10} BOC-aminoalkyl, and C_3-C_{10} cycloalkyl or are joined together to form a heterocyclic ring containing two to five carbon atoms, and R^c is selected from the group consisting of C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10} BOC-aminoalkyl, and C_3-C_{10} cycloalkyl;

- X_2 is H, hydroxyl, C_1-C_{10} alkylamino, C_1-C_{10} alkylamido or C_1-C_{10} hydroxyalkyl;
- X_3 and X_4 each independently are hydrogen, hydroxyl, amino, amido, azido, halo, alkyl, alkoxy, carboxy, nitrilo, nitro, trifluoro, aryl, alkaryl, thio, thioester, thioether, -OCOPh, -OC(=S)OPh or both X_3 and X_4 are oxygen connected to >C=S to form a 5-membered ring, or X_2 and X_3 form the ring of formula (III):



(III)

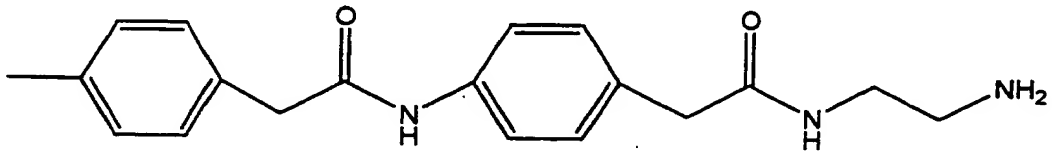
where R' and R" are independently C_1-C_{10} alkyl;

- R_2 is selected from the group consisting of hydrogen, halo, C_1-C_{10} alkylether, amino, hydrazido, C_1-C_{10} alkylamino, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, pyridylthio, C_2-C_{10} alkenyl; C_2-C_{10} alkynyl, thio, and C_1-C_{10} alkylthio; and

R_3 is a $-NR_4R_5$ group with R_4 being hydrogen or a group selected from alkyl, substituted alkyl or aryl-NH-C(Z)-, with Z being O, S, or NR^a with R^a having the above meanings,

- and R_5, where R_4 is hydrogen, is selected from the group consisting of R- and S-1-phenylethyl, benzyl, phenylethyl or anilide groups unsubstituted or substituted in one or more positions with a substituent selected from the group consisting of C_1-C_{10} alkyl, amino, halo, C_1-C_{10} haloalkyl, nitro, hydroxyl, acetoamido, C_1-C_{10} alkoxy, and sulfonic acid or a salt thereof; or R_4 is benzodioxanemethyl, fururyl, L-propylalanyl-

aminobenzyl, β-alanylamino- benzyl, T-BOC-β-alanylaminobenzyl, phenylamino, carbamoyl, phenoxy or C_1-C_{10} cycloalkyl; or R_5 is a group of the following formula:

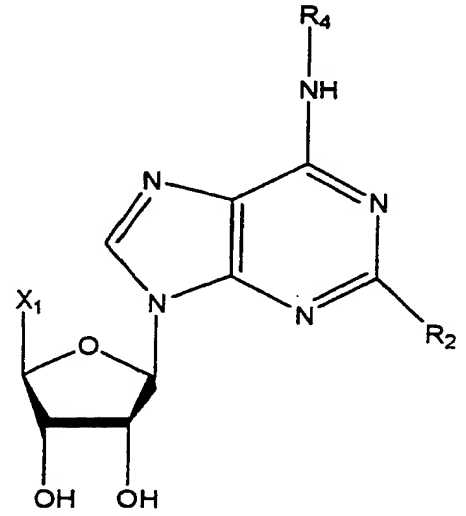


- or a suitable salt of the compound defined above, e.g. a triethylammonium salt thereof; or

when R_4 is, a group selected from alkyl, substituted alkyl, or aryl-NH-C(Z)-, then, R_4 is selected from the group consisting of substituted or unsubstituted heteroaryl-NR^a-C(Z)-, heteroaryl-C(Z)-, alkaryl-NR^a-C(Z)-, alkaryl-C(Z)-, aryl-NR-C(Z)- and aryl-C(Z)-;

wherein Z having the above defined meanings.

5. Use according to Claim 3, wherein said active ingredient is a nucleoside derivative of the general formula (IV):



(IV)

wherein X_1, R_2 and R_4 are as defined in Claim 3.

6. Use according to claim 5, wherein said active ingredient is an N6-benzyladenosine-5′-uronamide derivative.

7. Use according to Claim 6, wherein said active ingredient is selected from N^6-2-(4-aminophenyl)ethyladenosine (APNEA), N^6-(4-amino-3- iodobenzyl) adenosine-5'-(N-methyluronamide) (AB-MECA) and 1-deoxy-1-{6- [({3-iodophenyl} methyl)amino]- 9H-purine-9-yl}-N-methyl- β-D-ribofuranuron-amide (IB-MECA) and 2-chloro-N^6-(2-iodobenzyl)-adenosine- 5'-N-methly-uronamide (Cl-IB-MECA).

8. Use according to any one of Claims 1 to 7, wherein said therapeutic effect is to counter drug-induced myelotoxicity.

9. Use according to Claim 8, wherein said drug is a chemotherapeutic drug given to the subject within the framework of anti-cancer treatment.

10. Use of an effective amount of an active ingredient for the preparation of a pharmaceutical composition for achieving a therapeutic effect, the therapeutic effect comprises inducing proliferation or differentiation of bone marrow or white blood cells, wherein the active ingredient is selected from an adenosine A3 receptor agonist (A3RAg), an adenosine A2 receptor antagonist (A2RAn) or a combination of an A3RAg or an A2RAn.

11. Use according to Claim 10, wherein said active ingredient is A3RAg.

12. Use according to Claim 10, for the preparation of a pharmaceutical composition for oral administration.

13. Use according to any one of Claims 10-12, wherein the active ingredient is that defined in any one of Claims 4-7.

14. Use of an effective amount of an active ingredient for the preparation of a pharmaceutical composition for the prevention or treatment of leukopenia, the active ingredient being selected from an adenosine A3 receptor agonist (A3RAg), an adenosine A2 receptor antagonists (A2RAn) or a combination of an A3RAg or an A2RAn.

15. Use according to Claim 15, for the preparation of a pharmaceutical composition for the prevention or treatment of drug- induced leukopenia.

16. Use of an effective amount of an active ingredient for the preparation of a pharmaceutical composition for the prevention or treatment of toxic side effects of a drug, the active ingredient being selected from an adenosine A3 receptor

agonist (A3RAg), an adenosine A2 receptor antagonists (A2Ran) or a combination of an A3RAg and an A2RAn.

17. Use according to Claim 16, wherein the toxic side effect is manifested by weight loss.

18. Use according to any one of Claims 14-17, wherein said drug is a chemotherapeutic drug.

19. Use according to Claim 14, for the preparation of a pharmaceutical composition for achieving a therapeutic effect, the therapeutic effect comprises elevating the level of circulating leukocytes in the subject.

20. Use according to any one of Claims 14-19, wherein said active ingredient is A3RAg.

21. Use according to Claim 20, for the preparation of a pharmaceutical composition for oral administration.

22. Use according to Claim 21, wherein said active ingredient is that defined in any one of Claims 4-7.

23. Use of an active ingredient selected from an adenosine A3 receptor agonist (A3RAg), an adenosine A2RAn and a combination of an A3RAg and an A2RAn for the manufacture of a pharmaceutical composition, wherein said manufacture comprises mixing said active ingredient with a drug that can cause a toxic side effect in a treated subject.

24. Use according to Claim 23, wherein said drug is a chemotherapeutic drug.

25. Use according to Claim 23 or 24, for the preparation of a pharmaceutical composition for oral administration.

26. Use of an effective amount of an active ingredient for the preparation of a pharmaceutical composition for achieving a therapeutic effect, the therapeutic effect comprises inhibition of abnormal cell growth, the active ingredient being selected from an adenosine A3 receptor agonist (A3RAg), an adenosine A2 receptor agonist (A2RAg) or a combination of A3RAg and A2RAg.

27. Use according to Claim 26, wherein said therapeutic effect comprises inhibition of growth or proliferation of tumor cells.

28. Use according to Claim 26, wherein said active ingredient is an A3RAg.

29. Use according to Claim 26, for the preparation of a pharmaceutical composition for oral administration.

30. Use according to Claim 26, wherein said pharmaceutical composition is intended for administration in combination with a chemotherapeutic drug.

31. Use according to Claim 26, wherein said active ingredient is as defined in any one of Claims 4-7.

32. Use of an A3RAg for the preparation of a pharmaceutical composition for the treatment of cancer in a subject, the pharmaceutical composition yielding a dual effect in both inhibiting proliferation of cancer cells and countering toxic side effects of a chemotherapeutic drug treatment of the same subject.

33. Use according to Claim 32, wherein the active ingredient synergizes with said drug to yield a stronger anti-tumor effect.

34. Use according to Claim 32 or 33, wherein the active ingredient is formulated for oral administration.

35. Use according to any one of Claims 32-34, wherein the active ingredient is that defined in any one of Claims 4-7.

PCT REQUEST 125955.5 DAB

Original (for **SUBMISSION**) - printed on 07.09.2000 04:17:21 PM

0	**For receiving Office use only**	
0-1	International Application No.	
0-2	International Filing Date	
0-3	Name of receiving Office and "PCT International Application"	
0-4	**Form - PCT/RO/101 PCT Request**	
0-4-1	Prepared using	PCT-EASY Version 2.91 (updated 01.07.2000)
0-5	**Petition** The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	**Receiving Office (specified by the applicant)**	Israel Patent Office (RO/IL)
0-7	**Applicant's or agent's file reference**	125955.5 DAB
I	**Title of invention**	PHARMACEUTICAL COMPOSITIONS COMPRISING AN ADENOSINE RECEPTOR AGONIST OR ANTAGONIST
II	**Applicant**	
II-1	This person is:	applicant only
II-2	Applicant for	all designated States except US
II-4	Name	CAN-FITE TECHNOLOGIES LTD.
II-5	Address:	21 Achad Ha'am Street 61040 Tel Aviv Israel
II-6	State of nationality	IL
II-7	State of residence	IL
III-1	**Applicant and/or inventor**	
III-1-1	This person is:	applicant and inventor
III-1-2	Applicant for	US only
III-1-4	Name (LAST, First)	FISHMAN, Pnina
III-1-5	Address:	19 Asher Barash Street 46365 Herziliya Israel
III-1-6	State of nationality	IL
III-1-7	State of residence	IL

PCT REQUEST 125955.5 DAB

Original (for **SUBMISSION**) - printed on 07.09.2000 04:17:21 PM

IV-1	**Agent or common representative; or address for correspondence** The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	agent
IV-1-1	Name	REINHOLD COHN AND PARTNERS
IV-1-2	Address:	P.O.Box 4060 61040 TEL-AVIV Israel
IV-1-3	Telephone No.	+972-3-7109314
IV-1-4	Facsimile No.	+972-3-7109407
IV-1-5	e-mail	dabarash@cohnpatents.co.il
V	**Designation of States**	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW MZ SD SL SZ TZ UG ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE CH&LI CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH&LI CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
V-5	**Precautionary Designation Statement** In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.	
V-6	**Exclusion(s) from precautionary designations**	NONE

PCT REQUEST 125955.5 DAB

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VI-1	**Priority claim of earlier national application**		
VI-1-1	Filing date	10 September 1999 (10.09.1999)	
VI-1-2	Number	131864	
VI-1-3	Country	IL	
VI-2	**Priority claim of earlier national application**		
VI-2-1	Filing date	23 December 1999 (23.12.1999)	
VI-2-2	Number	133680	
VI-2-3	Country	IL	
VI-3	**Priority document request** The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s):	VI-1, VI-2	
VII-1	**International Searching Authority Chosen**	European Patent Office (EPO) (ISA/EP)	
VIII	**Check list**	number of sheets	electronic file(s) attached
VIII-1	Request	4	-
VIII-2	Description	41	-
VIII-3	Claims	11	-
VIII-4	Abstract	1	125955-5_db-tm_can_fite.txt
VIII-5	Drawings	15	-
VIII-7	TOTAL	72	
	Accompanying items	paper document(s) attached	electronic file(s) attached
VIII-8	Fee calculation sheet	✓	-
VIII-9	Separate signed power of attorney	✓	-
VIII-16	PCT-EASY diskette	-	diskette
VIII-18	**Figure of the drawings which should accompany the abstract**	NONE	
VIII-19	**Language of filing of the international application**	English	
IX-1	**Signature of applicant or agent**		
IX-1-1	Name	REINHOLD COHN AND PARTNERS	
IX-1-2	Name of signatory	COHN, Ilan	
IX-1-3	Capacity	Patent Attorney	

FOR RECEIVING OFFICE USE ONLY

10-1	**Date of actual receipt of the purported international application**	
10-2	**Drawings:**	
10-2-1	Received	
10-2-2	Not received	
10-3	**Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application**	
10-4	**Date of timely receipt of the required corrections under PCT Article 11(2)**	
10-5	**International Searching Authority**	ISA/EP

PCT REQUEST

125955.5 DAB

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10-6	Transmittal of search copy delayed until search fee is paid	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	Date of receipt of the record copy by the International Bureau	

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 March 2001 (22.03.2001)

PCT

(10) International Publication Number
WO 01/19360 A2

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(54) Title: PHARMACEUTICAL COMPOSITIONS COMPRISING AN ADENOSINE RECEPTOR AGONIST OR ANTAGONIST

(57) Abstract: Adenosine receptor agonists, particularly an agonist which binds to the A3 adenosine receptor, are used for induction of production or secretion of G-CSF within the body, prevention or treatment of toxic side effects of a drug or prevention or treatment of leukopenia, particularly drug-induced leukopenias; and inhibition of abnormal cell growth and proliferation.

PHARMACEUTICAL COMPOSITIONS COMPRISING AN ADENOSINE RECEPTOR AGONIST OR ANTAGONIST

FIELD OF THE INVENTION

The present invention is generally in the field of cancer and concerns a cancer therapy or a therapy intended to counter the side effect of cancer treatment.

PRIOR ART

The following is a list of prior art which is considered to be pertinent for describing the state of the art in the field of the invention. Acknowledgement of these references herein will be made by indicating the number from their list below within brackets.

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BACKGROUND OF THE INVENTION

Myelotoxicity is a prevailing, severe, complication of chemotherapy and is one of the factors that limit the administrable dose of the chemotherapeutic drug. It causes more life threatening patient morbidity and actual mortality than any other chemotherapeutic side effect and may result in an increased number of hospital stay days. In addition, drug induced myelosuppression limits the administration of larger, potentially more effective doses of chemotherapy to patients with malignancies. Several approaches to resolve this adverse event have included the use of lithium, prostaglandin E, interferon, lactoferrin and the growth factors granulocyte-macrophage colony stimulating factor (GM-CSF) and granulocyte-colony stimulating factor (G-CSF). To date, use of growth factors such as G-CSF is a standard therapy for cancer patients with neutropenia. It stimulates the proliferation and differentiation of hematopoietic progenitors and also controls the functional activities of neutrophils and macrophages. However, the G-CSF treatment is costly and as it is a recombinant protein, it has accompanying side effects.

Adenosine, an endogenous purine nucleoside, is ubiquitous in mammalian cell types. Adenosine present in the plasma and other extracellular fluids mediates many of its physiological effects via cell surface receptors and is an important regulatory protein. It is released into the extracellular environment from metabolically active or stressed cells. It is known to act through its binding to specific G-protein associated A1, A2 and A3 membranal receptors[(1-2)]. The interaction of adenosine with its receptors initiates signal transduction pathways, mainly the adenylate cyclase effector system, which utilizes cAMP as a second

messenger. While A1 and A3 receptors, which are coupled with Gi proteins, inhibit adenylate cyclase and lead to a decrease in the level of intracellular cAMP, the A2 receptor, which is coupled to Gs proteins, activates adenylate cyclase, thereby increasing cAMP levels[3].

Since specific surface receptors for adenosine are found in nearly all cells, almost all organ systems of the body are regulated to some extent by its local release. This includes regulation of the electrophysiological properties of the heart, sedation and suppression of neurotransmitter's release and regulation of rennin release and vascular tone in the kidney[4-7]. Adenosine exerts various effects on the immune system including anti-inflammatory activity through the inhibition of cytokine release, inhibition of platelet aggregation, induction of erythropoietin production and modulation of the lymphocyte function[8-10]. Further, adenosine was found to play a role in the modulation of some central nervous system (CNS) functions, in wound healing, in diuresis and in controlling pain. It was also demonstrated that adenosine is capable of inducing proliferation in a wide range of normal cell types[11-14]. This modulation of cell growth is likely mediated through the adenylate cyclase effector system described above.

In a recent study it was found that adenosine acts as a chemoprotective agent, which activity is likely related to its capability to stimulate bone marrow cell proliferation. Further, it was found that adenosine exerted an inhibitory effect on the proliferation of tumor cells, apparently through G0/G1 cell cycle arrest and reduction of the telomeric signal [17-18]. The dual effect has turned adenosine into an attractive concept for cancer treatment.

SUMMARY OF THE INVENTION

In accordance with the present invention it was found that adenosine A3 receptor agonists (A3RAg) have a dual effect in that they inhibit proliferation of malignant cells on the one hand, and counter toxic side effects of chemotherapeutic drugs on the other hand. Specifically, the A3RAg compounds inhibit proliferation and growth of tumor cells, synergize with an anti-tumor cytotoxic drug in reducing

the tumor load, induce proliferation and differentiation of bone marrow cells and white blood cells and counter toxic side effects of other drugs, particularly chemotherapeutic drugs. Furthermore, it was discovered in accordance with the invention that the A3RAg exerts these activities by a variety of forms of administration including parenteral administration and particularly oral administration. It was further found in accordance with the invention that some of the A3RAg activity may be mimicked by other agonists and antagonists of the adenosine A1 or A2 receptors: the adenosine A1 receptor agonists (A1RAg) shares with the A3RAg its ability to induce G-CSF secretion; adenosine A2 receptor agonist (A2RAg) shares with the A3RAg its ability to inhibit proliferation of malignant cells; and the adenosine A2 receptor antagonist (A2RAn) shares with the A3RAg its ability to counter toxic side effects of drugs, e.g. treat or prevent leukopenia.

The invention relates in its broadest sense, to the use of an active ingredient to yield one of the following therapeutic/biological effects: inducing production or secretion of G-CSF within the body; prevention or treatment of toxic side effects of a drug or prevention or treatment of leukopenia, particularly drug-induced leukopenia; and inhibition of abnormal cell growth and proliferation. The active ingredient may be an A3RAg or an agonist or antagonist of the adenosine receptor system which can yield one of these therapeutic effects, achieved by the use of the A3RAg.

Several embodiments are provided by the invention. The first embodiment, to be referred to herein as the *"G-CSF-inducing embodiment"* involves the use of an active ingredient, which may be an A3Rag or an A1Rag to yield secretion of the G-CSF within the body of a treated subject. G-CSF is known to stimulate proliferation and differentiation of hematopoietic progenitors and controls the functional activities of neutrophils and macrophages. Thus, a G-CSF-inducing agent such as those mentioned above, may have a high therapeutic value, for example, in countering (i.e. preventing, reducing or ameliorating) myelotoxicity.

Provided in accordance with this embodiment is a method for inducing G-CSF secretion within the body of a subject, comprising administering to the subject an effective amount of an active ingredient selected from the group consisting of A3RAg, an A1RAg and a combination of an A3RAg and an A1RAg. In accordance with this embodiment there is further provided a method for the therapeutic treatment, comprising administering to a subject in need an effective amount of said active ingredient for achieving a therapeutic effect, the therapeutic effect comprises induction of G-CSF production or secretion. Still further provided by this embodiment is use of said active ingredient for the manufacture of a pharmaceutical composition for inducing G-CSF secretion. Also provided by this embodiment is a pharmaceutical composition for inducing production or secretion of G-CSF within the body, comprising a pharmaceutically acceptable carried an effective amount of said active ingredient.

In accordance with another embodiment of the invention, to be referred to herein at times as the *"Leukopenia-prevention embodiment"* or more specifically as the *"neutropenia-prevention embodiment"*, an active ingredient which may be an A3RAg, or an A2RAn, is used for the prevention or treatment of leukopenia, which may result from myelotoxicity.

In accordance with this embodiment there is provided a method for inducing proliferation or differentiation of bone marrow or white blood cells in a subject, comprising administering to the subject an effective amount of an active ingredient selected from the group consisting of an A3RAg, an adenosine A2RAn and a combination of an A3RAg or an A2RAn. Also provided by this embodiment is a method for prevention or treatment of leukopenia, comprising administering to a subject in need an effective amount of said active ingredient. Further provided in accordance with this embodiment is use of said active ingredient for the manufacture of a pharmaceutical composition for inducing proliferation or differentiation of bone marrow or white blood cells. Still further provided in accordance with this embodiment is use of said active ingredient for the manufacture of a pharmaceutical composition for the prevention or treatment of

leukopenia. The pharmaceutical composition can particularly be used for prevention or treatment of leukopenia.

In accordance with a related embodiment, to be referred to herein as the *"toxicity-preventing embodiment"* the abovementioned active ingredient (namely one of the A3RAg, or A2RAn, as well as a combination thereof, is used to counter toxic side effects of drugs, such as chemotherapeutic drugs or nemoleptic drugs .

In accordance with this latter embodiment there is thus provided a method for prevention or treatment of toxic side effects of a drug, comprising administering to a subject in need an effective amount of an active ingredient selected from the group consisting of an A3RAg, an A2RAn and a combination of an A3RAg and an A2RAn. Also provided in accordance with this embodiment is use of said active ingredient for the manufacture of a pharmaceutical composition for the prevention or treatment of drug-induced toxicity. Still further provided by this embodiment is pharmaceutical composition for prevention or treatment of toxic side effects of a drug, comprising an effective amount of said active ingredient and a pharmaceutically acceptable carrier.

For the purpose of countering drug-induced leukopenia or drug-induced toxic side effects in general, it is at times desirable to formulate a drug which has such toxic side effects together with said active ingredient for combined administration of the two. The invention thus also provides a pharmaceutical composition comprising, in combination a drug that can cause toxic side effect in a subject treated thereby and said active ingredient; as well as use of said active ingredient for the manufacture of such a pharmaceutical composition. Said active ingredients included in said composition being an amount effective for prevention or treatment of the toxic side effects.

In accordance with yet another embodiment of the invention, to be referred to herein as the *"proliferation-inhibiting embodiment"*, an active ingredient, which may be an A3RAg, an A2RAg, or a combination of the two, is used for selectively inhibiting abnormal cell growth, e.g. tumor cell growth.

In accordance with this embodiment there is provided a method for inhibiting abnormal cell growth in a subject, comprising administering to the subject a therapeutically effective amount of an active ingredient selected from the group consisting of an A3RAg, an A2RAg and a combination of an A3RAg and an A2RAg. Also provided in accordance with this embodiment is use of said active ingredient for the manufacture of a pharmaceutical composition for inhibiting abnormal cell growth. Still further provided by this embodiment is a pharmaceutical composition for inhibiting abnormal cell growth, comprising said active ingredient, and a pharmaceutically acceptable carrier.

In one embodiment of the invention the administration of the active ingredient is intended to achieve dual therapeutic effect: inhibition of abnormal cell growth and reduction of toxic side effects of a drug causing such effects.

The preferred active ingredient in accordance with the invention is an A3RAg. The preferred route of administration of the active ingredient, in accordance with the invention is the oral administration route. However, this preference does not exclude other active ingredients neither other administration routes of the active ingredients.

The dosage of the active ingredient, particularly where the active ingredient is an A3Rag, is preferably less than 100 μg/kg body weight, typically less than 50 μg and desirably within the range of 1-10 μg/kg body weight.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the invention novel therapeutic use is provided for certain active agents, particularly adenosine receptor agonists and antagonists. By one embodiment, the *G-CSF-inducing embodiment*, some such agents are used to mediate the production and secretion of G-CSF from cells. In accordance with another embodiment, the toxicity-preventing embodiment, some such agents are used to counter toxic side effects of drugs, e.g. chemotherapeutic or nemoleptic drugs. In a further embodiment, the *leukopenia-prevention embodiment*, some such agents are used to counter leukopenia, particularly drug-induced leukopenia.

In accordance with yet another embodiment, the *proliferation-inhibition embodiment*, some such agents are used to selectively inhibit abnormal cell growth.

The term "*leukopenia*" as used herein refers to the reduction in the circulating white blood cell count. While leukopenia is usually characterized by a reduced number of blood neutrophils (neutropenia), at times, a reduced number of lymphocytes, monocytes, eosinophils or basophils may be detected.

Leukopenia which may arise from the reduced production or excessive splenic sequestration of neutrophils, may result from a hereditary and congenital diseases. However it is mainly observed after treatment with drugs, such as cytoreductive cancer drugs, antithyroid drugs, phenothiazines, anticonvulsants, penicillins, sulfonamides, and chloramphenicol. Some antineoplastics cause leukopenia as a predictable side effect.

In the following, a reduction in leukocyte count or neutrophil count by drugs will be referred to herein, as "*drug-induced leukopenia*" or "*drug-induced neutropenia*". Furthermore, whenever mention is made to leukopenia, it should be understood as referring particularly to "*neutropenia*".

Further, the term *"prevention or treatment of leukopenia"* should be understood as a procedure whereby the reduction in leukocyte cell count which may otherwise occur, is reduced, totally prevented or if such reduction has occurred, a procedure which gives rise to increase in the leukocyte cell count. Leukopenia is manifested by a variety of side effects such as an increased possibility to infection by significant infectious agents and others. The term *"prevention or treatment of leukopenia"* should also be understood as meaning an improvement in such parameters which may occur as a result of leukopenia.

The pharmaceutically or therapeutically "*effective amount*" for purposes herein is determined by such considerations as may be known in the art. The amount must be effective to achieve the desired therapeutic effect which depends on the type and mode of treatment. As is clear to the artisan, the amount should be effective to obtain the improvement of survival rate, to obtain a more rapid

recovery. to obtain the improvement or elimination of symptoms or any other indicators as are selected as appropriate measures by those skilled in the art. When, for example, said active ingredient is administered to induce G-CSF production, an effective amount of the active ingredient may be an amount which leads to production and secretion of G-CSF from peripheral blood mononuclear cells, endothelial cell or fibroblast, in which it was produced, thereby, for example, stimulating the maturation of granulocytes progenitors into mature neutrophils. Where the active ingredient is administered to counter drug-induced leukopenia, an effective amount of the active ingredient may be an amount which protects the individual against the drug-induced reduction in the count of leukocytes, particularly neutrophils; an amount of the active ingredient which can give rise to an increase in an already decreased level of such cells, e.g. restore the level to a normal level or sometimes even above; etc. Where the active ingredient is administered in order to reduce toxic side effect of a drug, the amount of the active ingredient may, for example, be an amount effective in reduction of weight loss resulting from the drug administered. Where the active ingredient is administered in order to inhibit abnormal cell growth, as detailed hereinafter, an effective amount may be an amount which will inhibit the proliferation of such cells in the treated subject and even eliminate the tumor. Where the active ingredient is administered in order to potentiate the effect of an anti-cancer chemotherapeutic drug, an effective amount may be an amount which either increases the cancer specific toxicity of the chemotherapeutic treatment; an amount which is effective in reducing the amount of the chemotherapeutic drug or drug combination required to achieve a desired effect of the chemotherapeutic drug or drug combination, i.e. reduction of the tumor load; etc. An example of an effective amount is a daily administration of A3RAg less than 100 μg/kg body weight, typically less than 50 μg/kg body weight and optionally even less than 10 μg/kg body weight, e.g. about 3-6 μg/kg body weight. Such an amount of A3RAg is typically administered in a single daily dose although at times a daily dose may be divided into several doses administered throughout the day or at times several daily doses may be

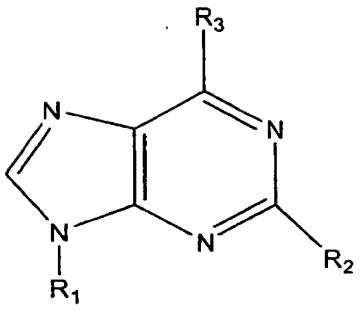
combined into a single dose to be given to the patient once every several days, particularly if administered in a sustained release formulation.

The active ingredient according to the invention is preferably an A3RAg. The A3RAg is any agonist which binds to A3 receptors and activates them to yield a therapeutic effect of the present invention. It should be noted that at times, an A3RAg may also interact with other receptors, e.g. with the A1 and A2 receptors. However, the A3RAg used in accordance with the invention exerts its prime effect through the A3 receptor (namely there may also be minor effects exerted through interaction with other adenosine receptors).

By one embodiment, the active ingredient according to the invention is a nucleoside derivative. By the term "*nucleoside*" it is meant any compound comprising a sugar, preferably ribose or deoxyribose, or a purine or pyrimidine base or a combination of a sugar with a purine or pyrimidine base preferably by way of N-glycosyl link. The term "*nucleoside derivative*" will be used to denote herein a naturally occurring nucleoside as define hereinabove, a synthetic nucleoside or a nucleoside which underwent chemical modifications by way of insertion/s, deletion/s or exocyclic and endocyclic substitution/s of group/s therein or conformational modifications which provide a derivative with the desired biological effect.

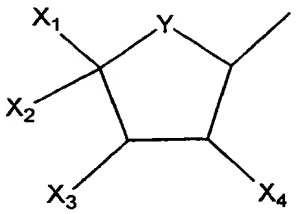
In accordance with one preferred embodiment of the invention the active ingredient is an A3RAg.

According to one embodiment of the invention, the active ingredient is a nucleoside derivative of the following general formula (I):



(I)

wherein R_1 is C_1-C_{10} alkyl, C_1-C_{10} hydroxyalkyl, C_1-C_{10} carboxyalkyl or C_1-C_{10} cyanoalkyl or a group of the following general formula (II):

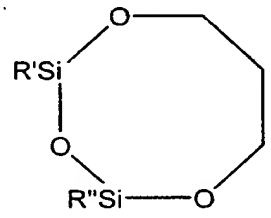


(II)

in which:

- Y is oxygen, sulfur of carbon atoms;
- X_1 is H, C_1-C_{10} alkyl, $R^aR^bNC(=O)$- or HOR^c-, wherein R^a and R^b may be the same or different and are selected from the group consisting of hydrogen, C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10} BOC-aminoalkyl, and C_3-C_{10} cycloalkyl or are joined together to form a heterocyclic ring containing two to five carbon atoms, and R^c is selected from the group consisting of C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10} BOC-aminoalkyl, and C_3-C_{10} cycloalkyl;
- X_2 is H, hydroxyl, C_1-C_{10} alkylamino, C_1-C_{10} alkylamido or C_1-C_{10} hydroxyalkyl;

- X_3 and X_4 each independently are hydrogen, hydroxyl, amino, amido, azido, halo, alkyl, alkoxy, carboxy, nitrilo, nitro, trifluoro, aryl, alkaryl, thio, thioester, thioether, -OCOPh, -OC(=S)OPh or both X_3 and X_4 are oxygen connected to >C=S to form a 5-membered ring, or X_2 and X_3 form the ring of formula (III):



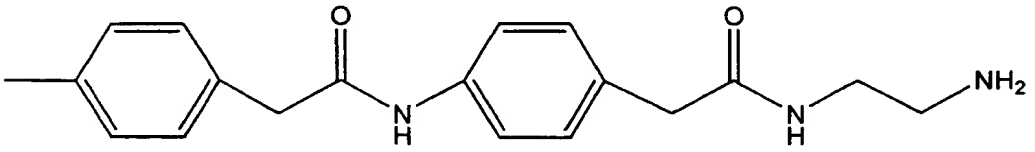
(III)

where R' and R" are independently C_1-C_{10} alkyl;

- R_2 is selected from the group consisting of hydrogen, halo, C_1-C_{10} alkylether, amino, hydrazido, C_1-C_{10} alkylamino, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, pyridylthio, C_2-C_{10} alkenyl; C_2-C_{10} alkynyl, thio, and C_1-C_{10} alkylthio; and

- R_3 is an $-NR_4R_5$ group, with R_4 being hydrogen or a group selected from alkyl, substituted alkyl or aryl-NH-C(Z)-, with Z being O, S, or NR^a with R^a having the above meanings,

- and R_5, where R_4 is hydrogen, is selected from the group consisting of R- and S-1-phenylethyl, benzyl, phenylethyl or anilide groups unsubstituted or substituted in one or more positions with a substituent selected from the group consisting of C_1-C_{10} alkyl, amino, halo, C_1-C_{10} haloalkyl, nitro, hydroxyl, acetoamido, C_1-C_{10} alkoxy, and sulfonic acid or a salt thereof; or R_4 is benzodioxanemethyl, fururyl, L-propylalanylaminobenzyl, β-alanylamino- benzyl, T-BOC-β-alanylamino-benzyl, phenylamino, carbamoyl, phenoxy or C_1-C_{10} cycloalkyl, or R_5 is a group of the following formula:

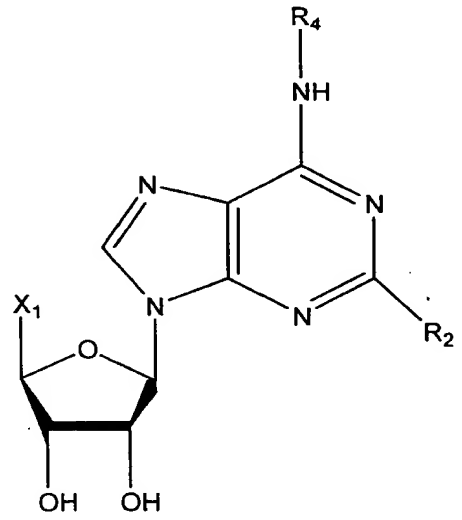


or a suitable salt of the compound defined above, e.g. a triethylammonium salt thereof; or

when R_4 is, a group selected from alkyl, substituted alkyl, or aryl-NH-C(Z)-, then, R_5 is selected from the group consisting of substituted or unsubstituted heteroaryl-NR^a-C(Z)-, heteroaryl-C(Z)-, alkaryl-NR^a-C(Z)-, alkaryl-C(Z)-, aryl-NR-C(Z)- and aryl-C(Z)-;

wherein Z having the above defined meanings.

According to this embodiment of the invention, the active ingredient is preferably a nucleoside derivative of the general formula (IV):



(IV)

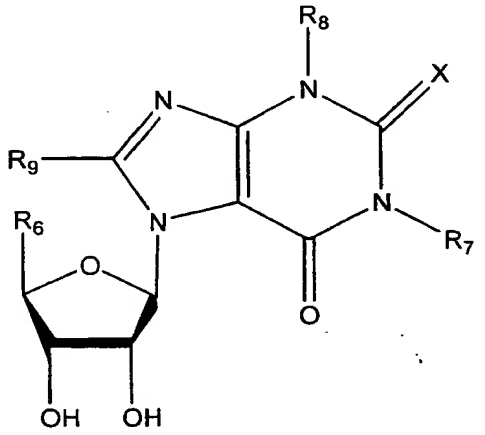
wherein X_1, R_2 and R_4 are as defined above and

Preferred active ingredients according to this embodiment of the invention may generally be referred to as N6-benzyladenosine-5'-uronamides and derivatives thereof found to be A3-selective adenosine receptor agonists. Examples for such derivatives are N^6-2-(4-aminophenyl)ethyladenosine (APNEA), N^6-(4-amino-3-iodobenzyl) adenosine-5'-(N-methyluronamide) (AB-MECA) and 1-deoxy-1-{6-[({3-iodophenyl} methyl)amino]- 9H-purine-9-yl}-N-methyl-β-D-ribofuranuron-amide the latter also referred to in the art as N^6-3-idobenzyl -5'-methylcarboxamidoadenosine, N6-(3-idobenzyl) adenosine-5'-N-methyl-

uronamide and herein above and below by the abbreviation IB-MECA or a chlorinated derivative of IB-MECA (R_2=Cl), referred to herein as Cl-IB-MECA, IB-MECA and Cl-IB-MECA being currently particularly preferred.

According to another embodiment of the invention, the active ingredient may be adenosine derivative generally referred to as N^6-benzyl-adenosine-5'-alkyluronamide-N^1-oxide or N^6-benzyladenosine-5'-N- dialyluron-amide -N^1-oxide.

Yet further, the active ingredient may be a xanthine-7-riboside derivative of the following general formula (V):



(V)

wherein:

- X is O or S;
- R_6 is $R^aR^bNC(=O)$- or HOR^c-, wherein
- R^a and R^b may be the same or different and are selected from the group consisting of hydrogen, C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, and C_3-C_{10} cycloalkyl, or are joined together to form a heterocyclic ring containing two to five carbon atoms; and
- R^c is selected from C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10} BOC-aminoalkyl and C_3-C_{10} cycloalkyl;
- R_7 and R_8 may be the same or different and are selected from the group consisting of C_1-C_{10} alkyl, C_1-C_{10} cycloalkyl, R- or S-1-phenylethyl,

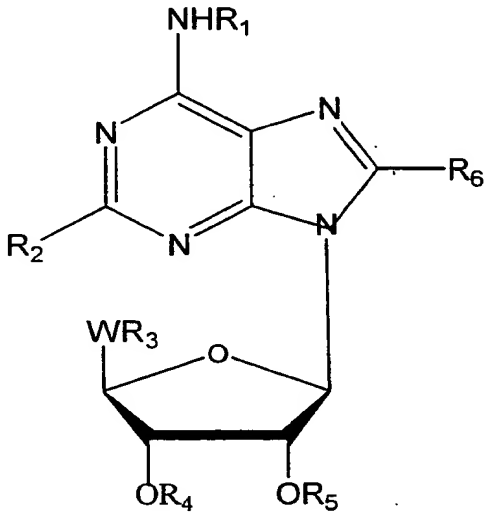
an unsubstituted benzyl or anilide group, and a phenylether of benzyl group substituted in one or more positions with a substituent selected from the group consisting of C_1-C_{10} alkyl, amino, halo, C_1-C_{10} haloalkyl, nitro, hydroxyl, acetamido, C_1-C_{10} alkoxy, and sulfonic acid;

- R_9 is selected from the group consisting of halo, benzyl, phenyl, C_3-C_{10} cyclalkyl, and C_1-C_{10} alkoxy;

or a salt of such a compound, for example, a triethylammonium salt thereof.

Some of the above defined compounds and their synthesis procedure may be found in detail in US 5,688,774; US 5,773,423, US 6,048,865, WO 95/02604, WO 99/20284 and WO 99/06053, incorporated herein by reference.

The active ingredient in the case of the GSF-inducing embodiment may also be an A1RAg. It is typically an adenosine derivative having the following formula

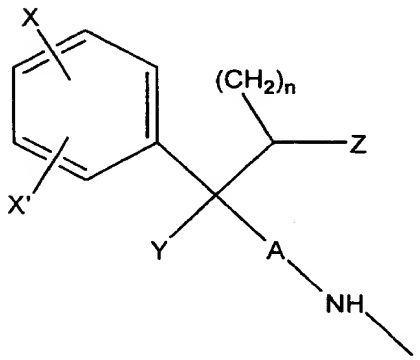


(VI)

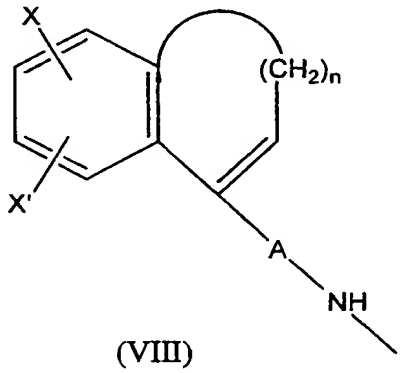
- R_1 represents a lower alkyl, cycloalkyl, preferably C_3-C_8 cycloalkyl (including the well known cyclohexyl and cyclopentyl containing derivatives, recognized as CPA and CHA, respectively), the cycloalkyl group may be substituted with, for example, a hydroxyl or lower alkyl; R_1 also represents a hydroxyl or hydroxyalkyl; a phenyl, anilide, or lower alkyl phenyl, all optionally substituted by one or more substituents, for example,

halogen, lower alkyl, haloalkyl such as trifluoromethyl, nitro, cyano, $-(CH_2)_mCO_2R^a$, $-(CH_2)_mCONR_2R^aR^b$, $-(CH_2)_mCOR^a$, m representing an integer from 0 to 6; $-SOR^c$, $-SO_2R^c$, $-SO_3H$, $-SO_2NR^aR^b$, $-OR^a$, $-SR^a$, $-NHSO_2R^c$, $-NHCOR^a$, $-NR^aR^b$ or $-NHR^aCO_2R^b$; wherein

- R^a and R^b represent independently a hydrogen, lower alkyl, alkanoyl, phenyl or naphthyl (the latter may be partially saturated) the alkyl group optionally being substituted with a substituted or unsubstituted phenyl or phenoxy group; or when R_1 represents $-NR^aR^b$, said R^a and R^b form together with the nitrogen atom a 5- or 6- membered heterocyclic ring optionally containing a second heteroatom selected from oxygen or nitrogen, which second nitrogen heteroatom may optionally be further substituted by hydrogen or lower alkyl; or $-NR^aB^b$ is a group of general formulae (VII) or (VIII):



(VII)



(VIII)

wherein n is an integer from 1 to 4;

- Z is hydrogen, lower alkyl or hydroxyl;
- Y is hydrogen, lower alkyl, or OR' where R' is hydrogen, lower alkyl or lower alkanoyl;
- A is a bond or a lower alkylene, preferably, C_1-C_4 alkenyl; and
- X and X' are each independently hydrogen, lower alkyl, lower alkoxy, hydroxy, lower alkanoyl, nitro, haloalkyl such as trifluoromethyl, halogen, amino, mono- or di-lower alkyl amino, or when X and X' are taken together a methylenedioxy group;
- R^c represents a lower alkyl;
- R_2 represents a hydrogen; halogen; substituted or unsubsituted lower alkyl or alkenyl group; lower haloalkyl or haloalkenyl; cyano; acetoamido; lower alkoxy; lower alkylamino; NR^dR^e where R^d and R^e are independently hydrogen, lower alkyl, phenyl or phenyl substituted by lower alkyl, lower alkoxy, halogen or haloalkyl such as trifluoromethyl or alkoxyl; or $-SR^f$ where R^f is hydrogen, lower alkyl, lower alkanoyl, benzoyl or phenyl;
- W represents the group $-OCH_2-$, $-NHCH_2-$, $-SCH_2-$ or $-NH(C=O)-$;
- R_3, R_4 and R_5 represent independently a hydrogen, lower alkyl or lower alkenyl, branched or unbranched C_1-C_{12} alkanoyl, benzoyl or benzoyl substituted by lower alkyl, lower alkoxy, halogen, or R_4 and R_5 form together a five membered ring optionally substituted by a lower alkyl or alkenyl; R_3 further represents independently a phosphate, hydrogen or dihydrogen phosphate, or an alkali metal or ammonium or dialkali or diammonium said thereof;
- R_6 represents a hydrogen, halogen atom; or
- one of the R groups (i.e. R_1 to R_6) is a sulfohydrocarbon radical of the formula R^g-SO_3-R^h-, wherein R^g represents a group selected from C_1-C_{10} aliphatic, phenyl and lower alkyl substituted aromatic group which may be substituted or unsubstituted and R^h represents a monovalent cation. Suitable monovalent cations include lithium, sodium, potassium, ammonium or trialkyl ammonium, which will enable dissociation to take

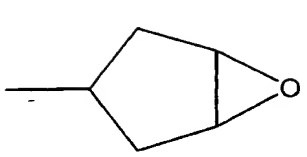
place under physiological conditions. The remaining R groups being a hydrogen or halogen atom, an unsubstituted hydrocarbon or any other non-sulfur containing group as defined above.

The hydrocarbon chains used herein may include straight or branched chains. In particular, the terms *"alkyl"* or *"alkenyl"* as used herein mean a straight or branched chain alkyl or alkenyl groups. The terms *"lower alkyl"* or *"lower alkenyl"* mean respectively C_1-C_{10} alkyl or C_2-C_{10} alkenyl groups and preferably, C_1-C_6 alkyl and C_2-C_6 alkenyl groups.

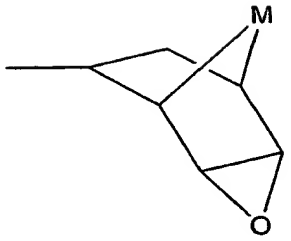
Preferred adenosine derivatives of formula (VI) are the N^6-cyclopentyl adenosine (CPA), 2-chloro-CPA (CCPA), and N^6-cyclohexyl adenosine (CHA) derivatives, the preparation of which is well known to the person skilled in the art. Other adenosine derivatives which are known to be selective to the A1 receptor are those wherein R_1 is a anilide group, the latter may be unsubstituted or substituted for example with hydroxyl, alkyl, alkoxy or with a group $-CH_2C(O)R''$, R" being an hydroxyl group, $-NHCH_3$, $-NHCH_2CO_2C_2H_5$,(ethyl glycinate), tuloidide (also in which the methyl moiety is replaced with a haloalkyl moiety), or with a group $-CH_2C(O)NHC_6H_4CH_2C(O)R'''$, in which R''' represents a group to yield a methyl ester substituent ($-OCH_3$), an amide substituent (e.g. R''' being a group $-NHCH_3$), or R''' being a hydrazide, ethylenediamine, $-NHC_2H_5NHC(O)CH_3$, 4-(hydroxy-phenyl)propionyl, biotinylated ethylene diamine or any other suitable hydrocarbon which renders the compound an A1 agonist.

Alternatively, the N^6-substituted adenosine derivatives used as active ingredients according to the present invention may be those containing an epoxide moiety and more particularly are a cycloalkyl epoxy containing adenosine derivative (e.g. oxabicyclo such as norbornanyl or oxatricyclo such as adamantanyl). Some such compounds may be defined by general formula (I),

wherein R_1 is a group of general formulae (IXa) and (IXb):



(IXa)



(IXb)

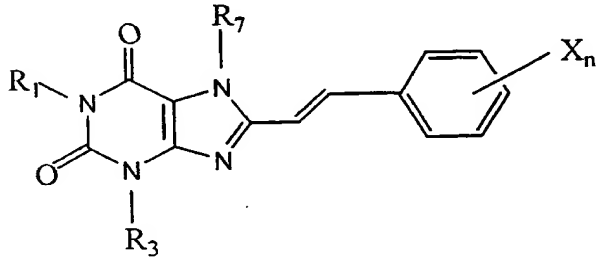
wherein M is a lower alkyl group as defined above.

Embodiments of the agonist compounds having an epoxide N^6-norbornyl group include the endo and exo isomers and more particularly, can be one of four isomers: the 2R-exo, 2R-endo, 2S-exo and 2S-endo form.

Another embodiment of the N^6-norbornyl derivative may include an oxygen atom at the N^1-position of the purine ring. This compound is termed N^6-(5,6-epoxynorborn-2-yl)adenosine-1-oxide.

At times, the A1RAg may be an adenine derivative in which the β-D-ribofuranozyl moiety of adenosine is replaced with a hydrogen or phenyl group.

A2RAn, which may be used in accordance with the invention are 8-styryl derivatives of 1,3,7-substituted xanthines of the formula (X):



wherein R_1 and R_3 are C_1-C_4 alkyl, allyl or propargyl

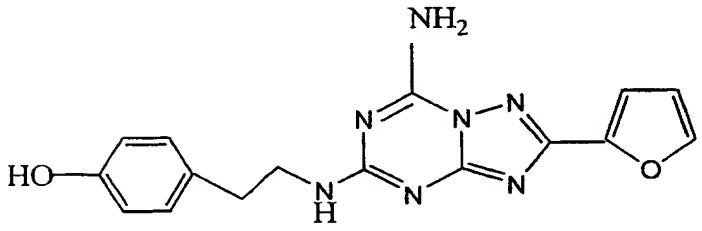
R_7 is H, methyl or C_2-C_8 alkyl

n is 1 to 3

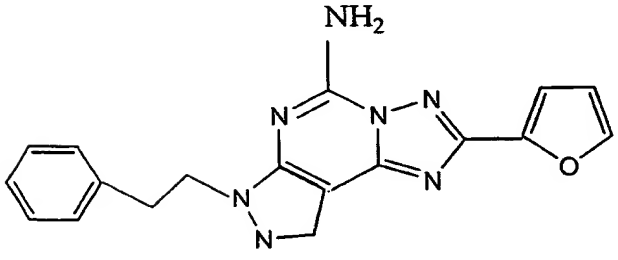
and X is a halogen, trifluoroalkyl, alkoxy, hydroxy, nitro, amino, dialkylamino, diazonium, isothiocyanate, benzyloxy, aminoalkoxy, alkoxy-carbonylamino, acetoxy, acetylamino, succinylamino, 4-(4-NH_2-trans-CH_2CH=$CHCH_2$0-3,5-$(MeO)_2$,4-(4-AcNH-trans-CH_2CH=$CHCH_2O$)-3,5-$(MeO)_2$, 4-(4-t-BOC-NH-trans-CH_2CH=$CHCH_2O$)-3,5-$(MeO)_2$

A specific example of the compound of formula (X) is (3,7-dimethyl-1-propargyl-xantane).

The A2RAn may also be compounds of the following formulae:



Or,



As will be appreciated, the invention may not be limited to the specific A3RAg, A2RAg or A2RAn compounds mentioned above.

The active ingredient in accordance with the invention may be as defined above or may be in the form of salts or solvates thereof, in particular physiologically acceptable salts and solvates thereof. Further, when containing one or more asymmetric carbon atoms, the active ingredient may include isomers and diastereoisomers of the above active ingredients or mixtures thereof.

Pharmaceutically acceptable salts of the above active ingredients include those derived from pharmaceutically acceptable inorganic and organic acids. Examples of suitable acids include hydrochloric, hydrobromic, sulphoric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicylic, succinic, toluene-p-sulfonic, tartaric, acetic, citric, methanesulfonic, formic, benzoic, malonic, naphthalene-2-sulfonic and benzenesulfonic acids.

The active ingredient may be administered as a non-active substance (e.g. pro-drug) and be made active only upon further modification/s by a natural process at a specific site in the subject. In any case, the derivative will be such that the therapeutic functionality of the pharmaceutical composition of the invention, is preserved. Such pro-drugs are also encompassed by the term *"active ingredient"* as used herein. Similarly, the terms *"A3RAg", "A1RAg" "A1RAn" "A2RAg"* and *"A2RAn"* should be understood as encompassing pro-drugs which, although *a priori*, lack the antagonistic or antagonistic activity (as the case may be), become active *in vivo*.

The A3RAg in accordance with the invention may be chosen by screening for such compounds which qualitatively have an activity resembling that of IB-MECA. For example, such compounds for use in accordance with the leukopenia-inhibiting embodiment may be screened based on their ability to stimulate proliferation of bone marrow or white blood cells and subsequently based on their ability to exert this activity *in vivo*. For use in the proliferation-inhibition embodiment, compounds may be screened for their ability to inhibit proliferation of tumor cells as well as subsequently to exert this activity *in vivo*.

The A1RAn and A2RAn may be tested for their activity and screened for use in therapy in a similar manner, *mutatis mutandis*, to that described for A3RAg.

The pharmaceutical composition of the invention may comprise the active ingredient as such, but may be combined with other ingredients which may be a pharmaceutically acceptable carrier, diluent, excipient, additive and/or adjuvent, as known to the artisan, e.g. for the purposes of adding flavors, colors, lubrication or the like to the pharmaceutical composition. Evidently, the pharmaceutically acceptable carrier/s, diluent/s, excipient/s, additive/s employed according to the invention generally refer to inert, non-toxic solid or liquid fillers, diluents or encapsulating materials which preferably do not react with the compounds within the composition of the invention.

Further, the active ingredient may also be administered in combination with a chemotherapeutic drug, particularly in the case of the leukopenia prevention embodiment. Thus the pharmaceutical composition according to the invention may comprise, in addition to said active ingredient a chemotherapeutic drug. According to one embodiment of the invention, the chemotherapeutic drug is an anti-cancer chemotherapeutic drug. It should be understood that by the term it is meant any cytotoxic drug or a cocktail comprising a combination of two or more cytotoxic drugs given to a patient for the purpose of reducing the patient's tumor mass.

One finding in accordance with the invention is that the A3RAg is orally bioavailable and exerts its dual activity (reducing abnormal cell proliferation and preventing or reducing leukopenia) when orally administered. Thus, according to one preferred embodiment, the pharmaceutical composition of the invention is formulated for oral administration. Such an oral composition may further comprise a pharmaceutically acceptable carrier, diluent, excipient, additive or adjuvant suitable for oral administration.

Within the scope of the G-CSF-inducing embodiment of the present invention, the pharmaceutical compositions disclosed are particularly used for increasing the level of G-CSF secreted from the cells. Such compositions may be used to accelerate the neutrophil recovery after chemotherapy and bone marrow transplantation or to inhibit abnormal cell growth. To date, such treatments include administration of the growth factor it self, which are known to have undesired side

effects. All the more so, the average cost per course of G-CSF therapy is known to be very high.

Within the scope of the leukopenia-prevention embodiment or the toxicity-preventing embodiment of the present invention, the pharmaceutical composition disclosed are particularly used for elevating the level of circulating leukocyte cells in a subject or countering other toxic effects, such as weight loss. This aspect of the invention is applicable in a variety of clinical situations. It is evident that a reduced level of circulating leukocytes and particularly neutrophils may result in a weakened immune system. An example of a weakened immune system which may be treated in accordance with this aspect of the invention, is such which often occurs in advanced stages of cancer or that resulting from drug-induced leukopenia or drug-induced neutropenia.

The proliferation-inhibiting embodiment is useful for the treatment of a variety of abnormalities associated with the abnormal cell growth such as cancer, psoriasis and some autoimmune diseases. In particular, the composition of the invention is employed for inhibiting proliferation of tumor cells, preferably within the framework of anti-cancer therapy.

When treating lymphoma cells with an A3RAg the inhibition of proliferation of these cells was more pronounced than that obtained with adenosine or the 'A1' or 'A2' agonists, although some activity was also observed with the A2RAg (see for example Fig. 5A). These results show that inhibition of tumor cell proliferation should be ascribed mainly to the binding of A3RAg to its corresponding receptor but may also be mimicked to some extent by an A2RAg. The above surprising results thus offers a new therapeutic target for future anti-cancer cytostatic drugs.

A3RAgs were further found to be potent in inhibiting growth of tumor cells, other than lymphoma, e.g. melanoma or colon carcinoma (see for example Fig. 6). A man versed in the art would clearly appreciate the advantage of treating a subject with a non-specific anti-cancer drug capable of inhibiting growth of the abnormally

dividing cells while concomitantly being capable restoring the immune system of the subject by inducing bone marrow cell proliferation.

Figs. 7A-7B, for example, show the differential effect of A3RAg. In this particular case, the effect of IB-MECA, on tumor and normal cells was evaluated. The more pronounced effect obtained using A3RAg, as compared to adenosine, is also clearly presented by these results. The therapeutic effect of A3RAg was reversed when an A3 receptor antagonist, MRS-1220, was employed.

The *in vivo* studies confirmed the *in vitro* results which demonstrated a chemoprotective effect of A3RAg on mice which were treated simultaneously with A3RAg and with a cytotoxic agent as compared to mice treated only with the cytotoxic drug (see for example Fig. 8). Further, a decrease in the number of foci in the A3RAg-treated mice was observed indicating the chemotherapeutic activity of A3RAg (see for example Fig. 9). Figures 10A-10B as well as 19A and 19B, for example, show that tumor-bearing mice treated only with the cytotoxic drug exhibited a decline in the number of peripheral blood leukocytes and neutrophils, while administration of A3RAg after chemotherapy, resulted in the restoration of the total white blood cell count yielding an increase in the percentage of neutrophils.

Thus, it may be concluded that A3RAg has a dual therapeutic function as it acts both as a chemotherapeutic agent as well as a chemoprotective agent. It is clear that use of A3RAg for this dual effect is also within the scope of the present invention.

In any case, the pharmaceutical compositions of the invention are administered and dosed in accordance with good medical practice, taking into account the clinical condition of the individual patient, the site and method of administration, scheduling of administration, patient's age, sex, body weight and other factors known to medical practitioners.

The composition of the invention may be administered in various ways. It can be administered orally, subcutaneously or parenterally including intravenous,

intraarterial, intramuscular, intraperitoneally or by intranasal administration, as well as by intrathecal and infusion techniques known to the man versed in the art.

As known, a treatment course in humans is usually longer than in animals, e.g. mice, as exemplified herein. The treatment has a length proportional to the length of the disease process and active agent effectiveness. The therapeutic regimen involved single doses or multiple doses over a period of several days or more. The treatment generally has a length contingent with the course of the disease process, active agent effectiveness and the patient species being treated.

When administering the compositions of the present invention parenterally, it will generally be formulated in a unit dosage injectable form (solution, suspension, emulsion). The pharmaceutical formulation suitable for injection includes sterile aqueous solutions or dispersions and sterile powders for reconstitution into sterile injectable solutions or dispersions. The carrier employed can be a solvent or dispersing medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, lipid polyethylene glycol and the like), suitable mixtures thereof and vegetable oils.

Non-aqueous vehicles such as cottonseed oil, sesame oil, olive oil, soybean oil, corn oil, sunflower oil, or peanut oil and ester, such as isopropyl myristate, may also at times be used as solvent systems for the active ingredient.

Additionally, various additives which enhance the stability, sterility and isotonicity of the compositions, including antimicrobial preservatives, antioxidants, chelating agents and buffers can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid and the like.

For the purpose of oral administration, the active ingredient may be formulated in the form of tablets, suspensions, solutions, emulsions, capsules, powders, syrups and the like, are usable and may be obtained by techniques well known to the pharmacists.

The present invention is defined by the claims, the contents of which are to be read as included within the disclosure of the specification, and will now be

described by way of example with reference to the accompanying Figures. It is to be understood, that the terminology which has been used is intended to be in the nature of words of description rather than limitation.

While the foregoing description describes in detail only a few specific embodiments of the invention, it will be understood by those skilled in the art that the invention is not limited thereto and that other variations in form and details may be possible without departing from the scope and spirit of the invention herein disclosed.

BRIEF DESCRIPTION OF THE FIGURES

In order to understand the invention and to see how it may be carried out in practice, a preferred embodiment will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Fig. 1 is a bar graph showing results of an *in vitro* assay in which the effect of adenosine (Ad), DPCPX (an A1RAn), CPA and CCPA (both A1RAg) or IB-MECA (an A3RAg) on G-CSF production is shown. Cultures treated with modified RPMI served as the control. The results are presented in terms of percent of control (control = 100%).

Fig. 2 is a bar graph showing results, obtained by [^{3}H]-thymidine incorporation assay, of an experiment in which stimulation of proliferation of bone marrow cells by either adenosine, CPA or IB-MECA, with ((+) G-CSF Ab – light-colored columns) or without antibodies against G-CSF ((-) G-CSF Ab – dark columns) was tested. The results show the neutralization effect of the anti-g-CSF antibodies. The results are represented in terms of percent increase over control (control = 0%).

Fig. 3A and **3B** are two bar graphs showing results, obtained by a [^{3}H]-thymidine incorporation assay, of an experiment, in which proliferation of bone marrow cells was tested in the presence of adenosine, an adenosine receptor agonists (Fig. 3A) or adenosine in combination with an adenosine receptor antagonists (Fig. 3B). The receptor agonists tested (Fig. 3A) are CPA (an A1RAg)

and IB-MECA (an A3RAg); the receptor antagonists tested (Fig. 3B) were DPCPX (an A1RAn), DMPX (an A2RAn) and MRS (an A3RAn). The results are presented in terms of percent increase in thymidine incorporation over control (control = 0%).

Fig. 4 is a bar graph showing results of an *in vitro* experiment in which the proliferation of bone marrow cells under three different concentrations of IB-MECA (0.01 μM, 0.1 μM and 1.0 μM) was tested. These results are presented in terms of the [^{3}H]-thymidine incorporation – percent above control (control = 0%). The numbers below the bars are the IB-MECA concentrations (μM).

Figs. 5A and **5B** are bar graphs showing results of two experiments, both carried out *in vitro* and being based on cell count assays, in which the effect of growth of lymphoma cells (Nb2-11C) by adenosine and its antagonist was tested. In the experiment shown in Fig. 5A, the effect on lymphoma cell growth by adenosine, CPA (an A1RAg), DMPA (an A2RAg) or IB-MECA (an A3RAg) was tested. In the experiment shown in Fig. 5B, the effect on the lymphoma cell growth by adenosine, DPCPX (an A1RAn), DMPX (an A2RAn) or MRS-1220 (an A3RAn) was tested. RPMI-treated lymphoma cells serve as control. The results are depicted as % inhibition of growth over that of control (control = 0%).

Fig. 6 is a bar graph showing results of an *in vitro* assay in which growth of different tumor cell types (B16 melanoma, HTC-116 colon carcinoma, Nb2-11C lymphoma) was inhibited in the presence of the A3RAg IB-MECA. RPMI-treated cells served as control. The results are presented as percent inhibition over control (control = 0%).

Fig. 7A and **7B** are bar graphs showing the results of an *in vitro* assay in which the effect of adenosine or the A3RAg, IB-MECA on growth of tumor cells (Nb2-11C Lymphoma, Fig. 7A) or bone marrow cells (Fig. 7B was tested). The results in Fig. 7A and 7B are shown in terms of percent inhibition and percent stimulation, respectively, as compared to control (control = 0%).

Fig. 8 is a bar graph showing the results of an *in vivo* experiment where the count of peripheral white blood cells (WBC) after 5 and 9 days of treatment with a

chemotherapeutic drug (cyclophosphamide) was tested. The cyclophosphamide was either administered alone (gray columns) or in combination with IB-MECA administered orally (in a 1 ml solution) by daily administration, beginning 24 hours after the chemotherapeutic drug. PBS-treated mice served as control. The WBC count (WBC Counts) is given as percent over control (control = 0%).

Fig. 9 is a bar graph showing results of an *in vivo* experiment in which the number of melanoma *foci* developed in mice following inoculation of $2x10^5$ melanoma cells into the mice, treated with chemotherapy cyclophosphamide (CHEMO), with IB-MECA, an A3RAg with a combination of IB-MECA and CHEMO or with phosphate buffer saline (PBS) which served as control.

Figs. 10A and **10B** are bar graphs showing the results of *in vivo* experiment demonstrating the chemotherapeutic activity of IB-MECA. The level of white blood cells (WBC, Fig. 10A) and neutrophils (Fig. 10B) as a function of time (hours after administration) of the chemotherapeutic drug cyclophosphamide (CHEMO) with (CHEMO + IB-MECA) and without IB-MECA administration is shown). Tumor bearing mice treated with PBS served as control. The neutrophil count is shown as % over control (control = 0%).

Fig. 11 shows weight of nude mice at 7, 10 and 14 days after onset of treatment (administration of 5-FU, Cl-IB-MECA or a combination of 5-FU and Cl-IB-MECA), as % of control (non-treated mice = 100%). The treatments consisted of administration of 5-FU (dark columns), administration of 5-FU in combination with Cl-IB-MECA (an A3RAg) – gray columns) and Cl-IB-MECA alone (white columns).

Fig. 12A and **12B** show results of an experiment in which the effect of Cl-IB-MECA in reduction of doxorubicin-induced myelotoxicity was examined. The experiment was performed in ICR mice. Fig. 12A shows the white blood cell (WBC) count while Fig. 12B shows the count of bone marrow nucleated cells. In Fig. 12A results are shown for the two different treatments at four different time periods, with the control level being indicated by a dashed line, while in Fig. 12B,

the results at two different time periods are shown with the control level being represented by a bar at the left hand side.

Fig. 13 shows the effect of anti-G-CSF antibodies on the number of white blood cells (WBC) in control mice, mice treated with a chemotheapeutic drug and mice treated with a chemotherapeutic drug and with Cl-IB-MECA, administered orally (6 μg/kg body weight, in 0.2 ml PBS). The number of WBC following injection of anti-G-CSF antibodies is represented by the light-colored columns. All results are presented as percent of control (control = 100%).

Fig. 14 shows the size of tumor, over time, developed in nude mice following injection of HCT-116 human colon carcinoma cells, in a control group and in a treated group (oral administration of Cl-IB-MECA).

Fig. 15 shows results of experiments similar to that of Fig. 14, where the size of the tumor developed in nude mice following injection of HCT-116 human colon carcinoma cells was measured. Four groups were tested: a control group, a group receiving the chemotherapeutic drug 5-FU, a group administered orally with Cl-IB-MECA and a group receiving a combined treatemnt of 5-FU and Cl-IB-MECA.

Fig. 16 is a bar graph showing the tumor size at day 30 in the experiment depicted in Fig. 15.

Fig. 17 is a bar graph showing results of an experiment where the Cl-IB-MECA-induced proliferation of bone marrow cells was measured under different concentrations (0.05 μg/ml and 0.5 μg/ml) of anti-G-CSF antibodies (0 – no antibodies). The proliferation was determined by [^{3}H]-thymidine incorporation assay.

Fig. 18 shows results of an *in vitro* experiment where proliferation of either B-16 melanoma or bone marrow cells was measured. The proliferation measured was the [^{3}H]-thymidine incorporation assay. The cells were exposed to either 0.01 μM and 0.1 μM Cl-IB-MECA with (white columns) or without (dark columns) the A3RAg, MRS-1523. The results are shown in terms of percent of control (control = 100%).

Figs. 19A and **19B** show results of an experiment similar to that shown in Figs. 10A and 10B, respectively, performed with Cl-IB-MECA.

Fig. 20 shows results of an *in vitro* experiment in which the proliferation of bone marrow cells induced by IB-MECA or Cl-IB-MECA was measured. These two A3RAg were added to the culture of the bone marrow cells at a concentration of either 1 nM or 10 nM, with (gray columns - *"(+) antagonists"*) or without (dark columns – *"(-) antagonists"*). An A3RAn, MRS-1523, at a concentration of 10 nM. The proliferation was determined by the [^{3}H]-thymidine incorporation assay. The results are given as percent stimulation versus control (untreated bone marrow cells, control = 0%).

EXPERIMENTAL RESULTS

Tumor cells

Murine tumor cell lines (B-16 melanoma and Nb2 11c rat Lymphoma) were used. B-16 melanoma cells were obtained from the American Type Tissue Culture Collection (ATCC), Rockville, Maryland. Nb2-11C rat lymphoma cells [Pines M., and Gertler A. *J. of Cellular Biochem.*, **37**:119-129 (1988)] was kindly provided by Dr. A. Gertler, Hebrew university, Israel.

Colon carcinoma cells (HCT-116) were also employed and were obtained at the ATCC.

The cells were routinely maintained in RPMI medium containing 10% fetal bovine serum (FBS, Biological Industries, Beit Haemek, Israel). Twice a week the cells were transferred to a freshly prepared medium.

Normal cells

Bone marrow cells derived from the femur of C57BL/6J mice were used. The cells were prepared as previously described [17].

Drugs/Compounds

The drugs employed were: adenosine; adenosine A1 receptor agonists: CCPA [2-chloro-N^6-cyclopentyl-adenosine], CPA (N-cyclopentyladenosine); A1RAn: DPCPX (1,3-dipropyl-8-cyclopentylxanthine); adenosine A2 receptor agonist: DMPA (N^6-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)-ethyl] adenosine) A2RAn: DMPX (3,7-dimethyl-1-propargyl-xantane); A3RAg: IB-MECA (1-deoxy-1-{6-[({3-iodophenyl}methyl)amino]-9H-purine-9-yl}-N-methyl-β-D-ribofuranuronamide)), CE-IB-MECA (2-chloro-N^6-3-iodobenzyl)-adenosine-5′-N-methyl-uronamide; and adenosine A3 receptor antagonist: MRS-1523 (5-propyl-2-ethyl-4-propyl-3-ethylsulfanylcarbonyl)-6-phenylpyridine-5-carboxylate) and MRS-1200 (9-chloro-2-(2-furanyl)-5-[(phenylacetyl)amino] [1,2,4,] –triazolo[1,5-c] quinazoline).

Anti-murine G-CSF antibodies (rabbit antiserum purified by protein A chromatography, Cytolab LTD, Weizmann Institute of Science, Israel) were used.

Cyclophosphamide was purchased from Taro Pharmaceutical Industries Ltd. Haifa Bay, Israel.

Mice

Female ICR, C57BL/6J or mice (BALB/C origin) mice aged 3 months, weighing an average of 25 gr were used. The mice were purchased from Harlan Laboratories, Jerusalem, ISRAEL. Standarized pelleted diet and tap water were supplied.

Example 1: Effect of adenosine and adenosine receptor antagonists and agonists on G-CSF production and bone marrow cell proliferation

To test the assumption that adenosine exerts its biological effect through stimulation of G-CSF production, normal cells were cultured in the presence adenosine or an adenosine agonist or antagonist.

For this purpose, bone marrow cells obtained from the femur of C57BL/6J or ICR mice were first disaggregated by passing through a 25G needle. Then, the cells ($3x10^5$cells/well, in 96 microtiter plates) were incubated with RPMI medium containing 10% fetal bovine serum (FBS) in the presence of adenosine (25μM). Adenosine or agonists to the A1 and A3 adenosine receptors - CPA (an A1RAg, 0.01μM), CCPA (an A1RAg, 0.01μM), or IB-MECA (an A3RAg, 0.01μM), were added to the bone marrow cultures in the absence of adenosine; an A1 adenosine receptor antagonist, DPCPX (0.1μM), was added to a bone marrow culture in the presence of adenosine (25μM).

Cultures containing cells suspended in RPMI medium and 5% FBS served as the control for the above detailed experiment.

[^{3}H]-Thymidine incorporation assay was used to evaluate the proliferation of the bone marrow cells. For this purpose, after 30 hours of incubation, each well was pulsed with 1μCi [^{3}H]-Thymidine. After a total of 48 hours of incubation, the cells were harvested and the [^{3}H]-Thymidine uptake was determined in an LKB liquid scintillation counter (LKB, Piscataway, NJ, USA). The results of this assay are depicted in Fig. 1 which shows that A1RAg or A3RAg have an effect on the production of G-CSF, that is similar to that obtained with adenosine.

To confirm that adenosine and its agonists exert their effect via stimulation of G-CSF production, a further assay was conducted where anti-G-CSF antibodies (62.5ng/ml) were added to a culture of bone marrow cells in the presence of adenosine (25μM), CPA (0.01μM) or IB-MECA (0.01μM). Cell proliferation was evaluated as described above. The results of this experiment are depicted in Fig. 2 which shows that antibodies to G-CSF inhibited the stimulatory effect of adenosine and its agonists on the proliferation of bone marrow cells. These results suggest that at least some of the activities associated with interaction with adenosine receptors is mediated through the induction of G-CSF.

The cumulative effect on the proliferation of bone marrow cells, when using a combination of an A1RAgm1 A3RAg, (CPA and IB-MECA) was evaluated. The assay was carried out similarly to that of the experiment the results of which are

shown in Fig. 1. Cells, after being disaggregated, were incubated in the presence of either adenosine (25μM), CPA (0.01μM), IB-MECA (0.01μM) or a combination of IB-MECA and CPA (each in a concentration of 0.01μM) and further treated as described above. The results are depicted in Fig. 3A which shows increased combined effect of IB-MECA and CPA

In order to compare the effect of adenosine receptor antagonist on the proliferation of bone marrow cells, following the same methodology described above, cells were incubated with adenosine alone or in combination with either DMPX (an A2RAn), DPCPX (an A1RAn), MRS-1220 (an A3RAn) or with a combination of DPCPX and MRS-1220. The results are shown in Fig. 3B. As can be seen, blocking the A2 receptor by DMPX also resulted in an increased proliferation of bone marrow cells which even exceeded that of adenosine alone. In comparison, proliferation with DPCPX or MRS-1220, reduced the increase by about 50% as compared to adenosine alone, while DPCPX in combination with MRS-1220 inhibited proliferation altogether.

Cells pre-treated as described above, were incubated at different concentrations of IB-MECA (1μM, 0.1μM or 0.01μM). The percent of stimulation was determined by [^{3}H]-Thymidine incorporation assay and the results are depicted in Fig. 3 which show that IB-MECA stimulates proliferation of bone marrow in a dose dependent manner.

Example 2: Modulation of tumor cell growth by adenosine and its agonists

Nb2-11C rat lymphoma cells ($1.2x10^4$ cells/ml) were incubated for 48 hours in 96 well microtiter plates with 1 ml RPMI medium containing 5% fetal bovine serum. Either 25μM adenosine, 0.01μM of an adenosine receptor agonists (CPA, an A1RAg; DPMA, an A2RAg or IB-MECA, an A3RAg) or 0.1μM of an adenosine receptor antagonists (DPCPX, an A1RAn; DMPX, an A2RAn; or MRS-1220, an A3RAn) in combination with adenosine (25μM) was added.

Cultures containing cells suspended in RPMI medium with 5% FBS served as controls for the above detailed experiment. Extent of cell proliferation was measured by a cell count assay.

The results are shown in Figs. 5A and 5B, comparable to the inhibition with adenosine. As can be seen, the proliferation of Nb2-11C cells, was markedly inhibited following incubation with IB-MECA, an A3RAg. No growth inhibition was seen in the presence of CPA, an A1RAg, and a lower growth inhibition was seen in the presence of DPMA, an A2RAn. The failure of CPA to inhibit the proliferation of these two tumor cells, suggested that the adenosine A1 receptor is not involved in this activity. However, the inhibitory activity of both DMPA and IB-MECA suggests the role of the A2 and the A3 adenosine receptors, respectively, in this inhibitory effect.

Further, it can be seen that DPCPX, an A1RAn, had essentially no effect, while in the presence of MRS-1220, an A3RAn, the effect of adenosine on the proliferation of Nb2-11C cells was substantially abolished. A minor, however still significant effect was exerted by DMPX, an A2RAn. These findings lead to the conclusion that tumor cell growth may be effectively inhibited by an A3RAg or an A2RAn.

In the same manner as described above, inhibition of growth of B-16 melanoma, HCT-116 colon carcinoma and Nb2-11C lymphoma, by the A3RAg, IB-MECA, was evaluated. The results are shown in Fig. 6 in terms of percent of inhibition or proliferation.

Example 3: Adenosine A3 receptor agonists exert a differential effect on tumor and normal cells

The effect of adenosine, A3RAns and A3RAgs, on the growth of tumor cells was examined, following the experimental procedure described above.

Briefly, Nb2-11C lymphoma or bone marrow cells were incubated in the presence of either adenosine, or IB-MECA. The dual effect of A3RAg is inhibiting

the growth of tumor cells while stimulating the proliferation of bone marrow cells is depicted Figs. 7A and 7B.

Example 4: ***In vivo*** **studies**

40 C57BL6/J mice were divided into 4 groups each of which were treated, by one of the following protocols:

1. Control group: daily intraperitoneal (i.p.) injection of 1 ml saline per mouse from day of tumor inoculation until the mice were sacrificed;
2. Chemotherapy group: one i.p. injection of cyclophsphoamide 24 hours after inoculation of tumor cells and daily i.p. injection of 1 ml saline per mouse from day of tumor inoculation until the mice were sacrificed.
3. Adenosine A3 receptor agonist (A3RAg) group: daily oral administration of IB-MECA from day of tumor inoculation until the mice were sacrificed.
4. A3RAg + chemotherapy group: one i.p. injection of cyclophsphoamide 24 hours after inoculation of tumor cells and daily oral administration of 3 μg/kg body weight of IB-MECA.

On day 5 and day 9 the mice were bled from the tail vain and blood samples were obtained for white blood cell (WBC) count. The results are depicted in Fig. 8.

In addition, following 18 days the mice were sacrificed and melanoma tumor foci were counted in the lung. The results are depicted in Fig. 9.

A further experiment was conducted in order to evaluate the chemoprotective effect of A3RAg. Mice were treated with cyclophosphoamide (50 mg/kg body weight in 0.3 ml PBS). After 48 and 72 hours from administration of the cytotoxic drug, the mice were injected i.p. with adenosine (25μg/kg body weight) or with IB-MECA (3 or 6 μg/kg body weight in 0.2 ml PBS) The number of white blood cells (WBC) and neutrophils was tested. The results are shown in Figs. 10A (WBCs) and 10B (neutrophils), respectively.

As can be seen, mice treated with cyclophosphoamide only exhibited a decline in the number of peripheral blood leukocytes and neutrophils as compared to the group treated only with IB-MECA. When adenosine or IB-MECA were administered, the total white blood cell count was restored with the latter having a very pronounced effect, yielding a complete recovery after 168 hours (7 days).

Example 5: Adenosine A3 receptor agonist prevents weight loss in mice treated with a chemotherapeutic drug

4 groups of nude mice (BALB/C origin), 10 in each group were treated as follows:

Group 1: The mice were untreated **[please confirm]**.

Group 2: The mice were injected intraperitoneally (i.p.) with 5-fluoro-uracyl (5-FU, 30 mg/kg body weight in PBS) for five consecutive days.

Group 3: The mice were injected i.p. with 5-FU as in Group 2 but starting on day 2, and every second day thereafter, the mice were given an oral administration of Cl-IB-MECA (6 μg/kg body weight, in 0.2 ml PBS.

Group 4: The mice received Cl-IB-MECA, as above.

The mice weight was measured at day 7, 10 and 14. The results are shown in Fig. 11.

As can be seen, 5-FU had a profound effect on the weight of the mice as compared to control, while Cl-IB-MECA administered together with the 5-FU, prevented some of this weight loss. The Cl-IB-MECA by itself did essentially not give rise to any weight loss.

This experiment demonstrates that the A3 adenosine receptor agonists have general protecting effect on some of the toxic effects of chemotherapy.

Example 6: Cl-IB-MECA protects the mice against myelotoxic effects of the chemotherapeutic drug doxorubicin

ICR mice were treated with doxorubicin (injection of 10 mg/kg i.p. in 0.5 ml PBS). After 24, 48 and 72 hours from administration of the cytotoxic drug, the mice were orally administered with Cl-IB-MECA (6 μg/kg body weight). At 72 hours, 96 hours, 120 hours and 144 hours, the mice were sacrificed and blood samples were withdrawn. In addition, bone marrow cells were aspirated from the femur of the mice and a cell count of nucleated cells from this aspirated preparation was made, following staining of the preparation with Coumassile Blue.

Three groups of mice were tested:

Group 1: (control) mice administered with PBS only.

Group 2: Mice treated with doxorubicin only.

Group 3: Administration of doxorubicin as above coupled with administration of Cl-IB-MECA).

The results of the white blood cell count can be seen in Fig. 12A, and that of the bone marrow nucleated cell count in Fig. 12B. These results clearly show that upon administration of Cl-IB-MECA, there is a marked increase in the number of peripheral white blood cells as well as in the number of bone marrow nucleated cells. This is evident to the protecting effect of the A3RAg against mylotoxic effects of doxorubicin.

Example 7: Antibodies against G-CSF neutralize the myeloprotectional effect of Cl-IB-MECA

ICR mice, were divided into six groups as follows:

Group 1: Control – administration of the vehicle only.

Group 2: Control with anti-G-CSF antibodies (5 μg/mouse).

Group 3: Chemotherapy – administration of cyclophosphoamide CYP – 50 mg/kg body weight).

Group 4: Chemotherapy (50 mg/kg body weight CYP) + anti-G-CSF antibodies (5 μg/mouse).

Group 5: Chemotherapy (50 mg/kg body weight CYP) + Cl-IB-MECA (6 μg/kg body weight) + anti-G-CSF antibodies (5 μg/mouse).

Group 6: Chemotherapy (50 mg/kg body weight CYP) + Cl-IB-MECA (6 μg/kg body weight) + anti-G-CSF antibodies (5 μg/mouse).

Each group consisted of 10 mice and the experiment was repeated twice.

The CYP was injected intraperitoneally in 0.2 ml of PBS which served as the carrier.

Cl-IB-MECA was given orally (in 0.2 ml PBS) at 48 hours and 72 hours following the administration of the cyclophophoamide.

Anti-G-CSF antibodies were intravenously injected (in 0.2 ml PBS) 72 hours following the administration of the chemotherapeutic drugs.

Blood samples were withdrawn 124 hours following chemotherapy. White blood cells (WBC) counts were made in a Coulter counter and differential cell counts were carried on smear preparations stained with May-Grundvald-Giemsa solution.

The results of the WBC count is shown in Fig. 13. As can be seen, mice treated with cyclophosphoamide only showed a decline in the number of peripheral blood WBC. In the group that was treated with Cl-IB-MECA, the WBC counts and the percentage of neutrophils were significantly higher in comparison to the chemotheapeutic treated group (results regarding transfer of neutrophils not shown). When anti-G-CSF antibodies were administered to the control or the chemotherapy groups, an expected decline in the number of WBC was observed. Administration of anti-G-CSF antibodies to the mice treated with the combination of the chemotherapeutic drug and Cl-IB-MECA, cancelled the protective effect of Cl-IB-MECA, as can clearly be seen in Fig. 13. These results lead to the conclusion that the protective effect of Cl-IB-MECA on the myeleoid

system is mediated through the ability of a Cl-IB-MECA to promote the production and secretion of G-CSF.

Example 8: Cl-IB-MECA inhibits the development of HCT-116 human coon carconima in nude mice

Tumors were established by subcutaneous injection of $1x10^6$ HCT-116 human colon cancer cells to nude mice (BALB/C origin) (Harlan, Jerusalem, Israel). Mice were treated orally with 6 μg/kg body weight Cl-IB-MECA (in 0.2 ml of PBS) every other day. Mice that were treated with the vehicle only (PBS). Each group consisted of 10 mice. Tumor growth rate was determined by measuring two orthogonal diameters of each tumor twice a week, and the tumor size was estimated according to the following formula: $\pi/6[D_1D_2]$. The results are depicted in Fig. 14. As can be seen, in the treated group there is a marked inhibition of tumor growth.

In a separate set of experiments a combined therapy of Cl-IB-MECA and 5-fluorouracyl (5-FU) was tested. $1x10^6$ HCT-116 cells were injected subcutaneously to nude mice. One day later, 5-FU (30 mg/kg body weight, in 0.2 ml PBS) was intraperitoneally injected and subsequently in 4 additional consecutive days. Every other day, the mice were administered orally with 5 μg/kg body weight of Cl-IB-MECA (in 0.2 ml of PBS). Mice that were treated either with the vehicle only (PBS) or with 5-FU served as control. Each group consisted of 10 mice. Tumor growth rate was determined by measuring two orthogonal diameters of each tumor twice a week, and the tumor size was estimated according to the following formula: $\pi/6[D_1D_2]$.

The results are depicted in Fig. 15 and 16. A marked inhibition of tumor growth was observed in the groups treated with 5-FU, Cl-IB-MECA and the combined therapy of Cl-IB-MECA and 5-FU. After 20 days a clear synergistic effect between Cl-IB-MECA and 5-FU in noting the tumor mass was seen, as depicted particularly in Fig. 16 (the results represented in Fig. 16 are those at day 30).

Example 9: Cl-IB-MECA stimulates bone marrow cell proliferation through the induction of G-CSF production

Bone marrow cells ($3x10^6$ celll/ml) were incubated in wells of 96 microtiter plates. Cl-IB-MECA at a final concentration of 10 nM was added with or without anti-G-CSF antibodies, at a final concentration of 0.05 and 0.5 μg/ml. Cell proliferation was measured by [^{3}H]-thymidine incorporation assay. The results are shown in Fig. 17.

As can be seen, the anti-G-CSF antibodies inhibit the proliferation of the bone marrow cells in a dose-dependent manner. This experiment also shows that the action of Cl-IB-MECA is mediated through the G-CSF pathway (involving G-CSF secretion from the cells).

Example 10: Cl-IB-MECA inhibits tumor cell growth and stimulates bone marrow proliferation and differentiation

B-16 melanoma cells ($5x10^5$ cells/ml) and bone marrow cells ($3x10^6$ cells/ml) were incubated in wells of 96 microtiter plate. The culture consisted of RPMI medium supplemented with 10% FTS: Cl-IB-MECA, at the concentration of 0.01 μM or 0.1 μM was added, with or without an antagonist of the adenosine A3 receptor, MRS-1523. Cell proliferation was measured by the [^{3}H]-thymidine incorporation assay mentioned before. The results are shown in Fig. 18. As can be seen, in the presence of MRS-1523, the proliferation of both the B-16 melanoma cells and the bone marrow cells was unchanged versus control. Against this, the Cl-IB-MECA exerted an inhibitory effect on proliferation of the B-16 melanoma cells, and a proliferation stimulation effect on the bone marrow cells.

These results demonstrate the dual effect of the A3 adenosine receptor agonists.

Example 11: Cl-IB-MECA acts as a chemoprotective agent

An example similar to that of Example 4, was performd with Cl-IB-MECA and the results are shown in Figs. 19A and 19B demonstrating the chemoprotective activity of Cl-IB-MECA.

Example 12: Effect of IB-MECA and Cl-IB-MECA on the proliferation of bone marrow cells

Murine bone marrow cells were cultured as described above. IB-MECA or Cl-IB-MECA were added to the cultures at a concentration of 1 or 10 nM, in the presence or absence of the A3RAn, MRS-1523. The antagonist was added at a concentration of 10 nM. The results are shown in Fig. 20.

As can be seen in Fig. 20, the effect of both IB-MECA and Cl-IB-MECA is dose dependent. Furthermore, as can also be seen, this effect is inhibited to a large extent by the A3RAn.

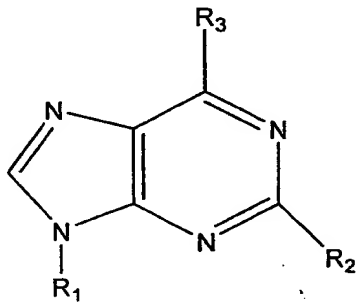
CLAIMS:

1. A method for inducing G-CSF secretion within the body of a subject, comprising administering to the subject an effective amount of an active ingredient selected from the group consisting of an adenosine A3 receptor agonist (A3RAg), an A1 adenosine receptor agonist (A1RAg) and a combination of an A3RAg and an A1RAg.

2. A method according to Claim 1, wherein said active ingredient is A3RAg.

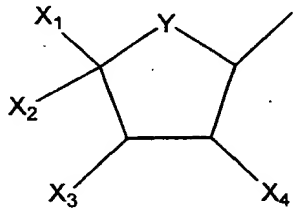
3. A method according to Claim 2, wherein the drug is administered orally.

4. method according to Claim 1, wherein said active ingredient is a nucleotide derivative of the following general formula (I):



(I)

wherein R_1 is C_1-C_{10} alkyl, C_1-C_{10} hydroxyalkyl, C_1-C_{10} carboxyalkyl or C_1-C_{10} cyanoalkyl or a group of the following general formula (II):



(II)

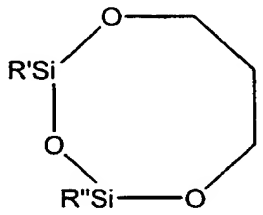
in which:

- Y is oxygen, sulfur of carbon atoms;
- X_1 is H, C_1-C_{10} alkyl, $R^aR^bNC(=O)$- or HOR^c-, wherein R^a and R^b may be the same or different and are selected from the group consisting of hydrogen, C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10}

BOC-aminoalkyl, and C_3-C_{10} cycloalkyl or are joined together to form a heterocyclic ring containing two to five carbon atoms, and R^c is selected from the group consisting of C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10} BOC-aminoalkyl, and C_3-C_{10} cycloalkyl;

- X_2 is H, hydroxyl, C_1-C_{10} alkylamino, C_1-C_{10} alkylamido or C_1-C_{10} hydroxyalkyl;

- X_3 and X_4 each independently are hydrogen, hydroxyl, amino, amido, azido, halo, alkyl, alkoxy, carboxy, nitrilo, nitro, trifluoro, aryl, alkaryl, thio, thioester, thioether, -OCOPh, -OC(=S)OPh or both X_3 and X_4 are oxygen connected to >C=S to form a 5-membered ring, or X_2 and X_3 form the ring of formula (III):



(III)

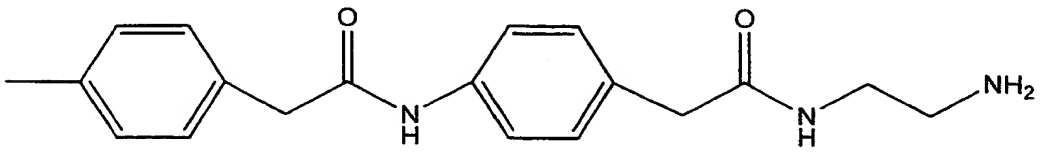
where R' and R" are independently C_1-C_{10} alkyl;

- R_2 is selected from the group consisting of hydrogen, halo, C_1-C_{10} alkylether, amino, hydrazido, C_1-C_{10} alkylamino, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, pyridylthio, C_2-C_{10} alkenyl; C_2-C_{10} alkynyl, thio, and C_1-C_{10} alkylthio; and

R_3 is a $-NR_4R_5$ group with R_4 being hydrogen or a group selected from alkyl, substituted alkyl or aryl-NH-C(Z)-, with Z being O, S, or NR^a with R^a having the above meanings,

- And R_5, where R_4 is hydrogen, is selected from the group consisting of R- and S-1-phenylethyl, benzyl, phenylethyl or anilide groups unsubstituted or substituted in one or more positions with a substituent selected from the group consisting of C_1-C_{10} alkyl, amino, halo, C_1-C_{10} haloalkyl, nitro, hydroxyl, acetoamido, C_1-C_{10} alkoxy, and sulfonic acid or a salt thereof; or R_4 is benzodioxanemethyl, fururyl, L-propylalanyl-

aminobenzyl, β-alanylamino- benzyl, T-BOC-β-alanylaminobenzyl, phenylamino, carbamoyl, phenoxy or C_1-C_{10} cycloalkyl; or R_5 is a group of the following formula:

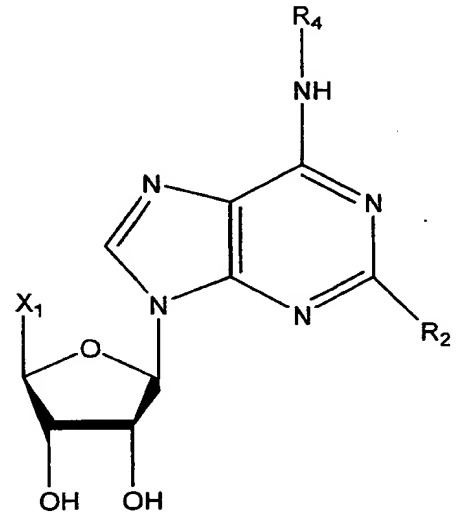


- or a suitable salt of the compound defined above, e.g. a triethylammonium salt thereof; or

when R_4 is, a group selected from alkyl, substituted alkyl, or aryl-NH-C(Z)-, then, R_4 is selected from the group consisting of substituted or unsubstituted heteroaryl-NR^a-C(Z)-, heteroaryl-C(Z)-, alkaryl-NR^a-C(Z)-, alkaryl-C(Z)-, aryl-NR-C(Z)- and aryl-C(Z)-;

wherein Z having the above defined meanings.

5. A method according to Claim 3, wherein said active ingredient is a nucleoside derivative of the general formula (IV):



(IV)

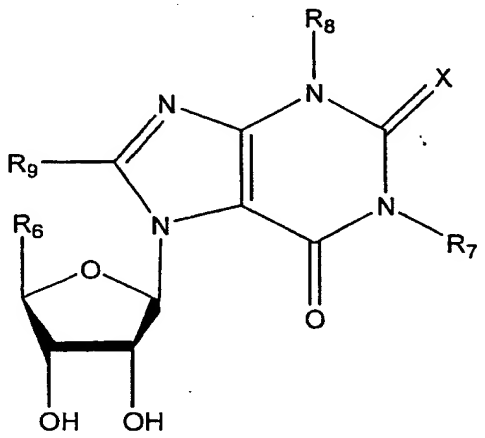
wherein X_1, R_2 and R_4 are as defined in Claim 3.

6. A method according to Claim 5, wherein said active ingredient is an N6-benzyladenosine-5′-uronamide.

7. A method according to Claim 6, wherein said active ingredient is selected from the group consisting of N^6-2-(4-aminophenyl)ethyladenosine (APNEA), N^6-(4-amino-3- iodobenzyl) adenosine-5'-(N-methyluronamide) (AB-MECA) and 1-deoxy-1-{6- [({3-iodophenyl} methyl)amino]- 9H-purine-9-yl}-N-methyl-β-D-ribofuranuron-amide (IB-MECA) and 2-chloro-N^6-(2-iodobenzyl)-adenosine-5′-N-methly-uronamide (Cl-IB-MECA).

8. A method according to Claim 1, wherein the active ingredient is N^6-benzyl-adenosine-5′-alkyluronamide-N^1-oxide or N^6-benzyladenosine-5′-N-dialyl-uron-amide-N^1oxide.

9. A method according to Claim 1, wherein the active ingredient is a xanthine-7-riboside derivative of the following general formula (VI):-



(VI)

wherein:

- X is O or S;
- R_6 is $R^aR^bNC(=O)$- or HOR^c-, wherein
  - R^a and R^b may be the same or different and are selected from the group consisting of hydrogen, C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, and C_3-C_{10} cycloalkyl, or are joined

together to form a heterocyclic ring containing two to five carbon atoms; and

- R^c is selected from C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10} BOC-aminoalkyl and C_3-C_{10} cycloalkyl;

- R_7 and R_8 may be the same or different and are selected from the group consisting of C_1-C_{10} alkyl, C_1-C_{10} cycloalkyl, R- or S-1-phenylethyl, an unsubstituted benzyl or anilide group, and a phenylether of benzyl group substituted in one or more positions with a substituent selected from the group consisting of C_1-C_{10} alkyl, amino, halo, C_1-C_{10} haloalkyl, nitro, hydroxyl, acetamido, C_1-C_{10} alkoxy, and sulfonic acid;

- R_9 is selected from the group consisting of halo, benzyl, phenyl, C_3-C_{10} cyclalkyl, and C_1-C_{10} alkoxy;

or a salt of such a compound, for example, a triethylammonium salt thereof.

10. A method for therapeutic treatment, comprising administering to a subject in need an effective amount of an active ingredient for achieving a therapeutic effect, the therapeutic effect comprises induction of G-CSF production or secretion, and said active ingredient selected from the group consisting of an adenosine A3 receptor agonist (A3RAg), an A1 adenosine receptor agonist (A1RAg) and a combination of an A3RAg and an A1RAg.

11. A method according to Claim 10, wherein said active ingredient is A3RAg.

12. A method according to Claim 11, wherein the drug is administered orally.

13. A method according to Claim 11 or 12, wherein said therapeutic effect is to counter drug-induced myelotoxicity.

14. A method according to Claim 13, wherein said drug is a chemotherapeutic drug given to the subject within the framework of anti-cancer treatment.

15. A method according to Claim 11, wherein the active ingredient is defined in any one of Claims 4-9.

16. Use of an active ingredient selected from the group consisting of an adenosine A3 receptor agonist (A3RAg), an A1 adenosine receptor agonist

(A1RAg) and a combination of an A3RAg and an A1RAg for the manufacture of a pharmaceutical composition for inducing G-CSF secretion.

17. Use according to Claim 16, wherein the active ingredient is an A3RAg.

18. Use according to Claim 17, wherein the drug is administered orally.

19. Use according to any one of Claims 16-18, wherein the active ingredient is that defined in any one of Claims 4-9.

20. A pharmaceutical composition for inducing production or secretion of G-CSF within the body, comprising a pharmaceutically acceptable carried an effective amount of an active ingredient selected from the group consisting of an adenosine A3 receptor agonist (A3RAg), an A1 adenosine receptor agonist (A1RAg) and a combination of an A3RAg and an A1RAg.

21. A pharmaceutical composition according to Claim 20, comprising an active ingredient as defined in any one of Claims 4-9.

22. A pharmaceutical composition according to Claim 20 or 21, for oral administration with said carrier being acceptable for oral administration.

23. A method for inducing proliferation or differentiation of bone marrow or white blood cells in a subject, comprising administering to the subject an effective amount of an active ingredient selected from the group consisting of an adenosine A3 receptor agonist (A3RAg), an adenosine A2RAn and a combination of an A3RAg or an A2RAn.

24. A method according to Claim 23, wherein said active ingredient is A3RAg.

25. A method according to Claim 24, wherein the drug is administered orally.

26. A method according to Claim 24, wherein the active ingredient is that defined in any one of Claims 4-9.

27. A method for prevention or treatment of leukopenia, comprising administering to a subject in need an effective amount of an active ingredient selected from the group consisting of an adenosine A3 receptor agonist (A3RAg), an A2RAn and a combination of an A3RAg or an A2RAn.

28. A method according to Claim 27, for prevention or treatment of drug-induced leukopenia.

29. A method for prevention or treatment of toxic side effects of a drug, comprising administering to a subject in need an effective amount of an active ingredient selected from the group consisting of an adenosine A3 receptor agonist (A3RAg), an adenosine A2 receptor antagonists (A2Ran) and a combination of an A3RAg and an A2RAn.

30. A method according to Claim 29, wherein the toxic side effect is manifested by weight loss.

31. A method according to Claims 28-30, wherein said drug is a chemotherapeutic drug.

32. A method according to Claim 27, for elevating the level of circulating leukocytes in the subject.

33. A method according to Claim 27 or 29, wherein said active ingredient is A3RAg.

34. A method according to Claim 33, wherein the drug is administered orally.

35. A method according to Claim 34, wherein the active ingredient is that defined in any one of Claims 4-9.

36. Use of an active ingredient selected from the group consisting of an adenosine A3 receptor agonist (A3RAg), an adenosine A2 receptor antagonist (A2RAn) and a combination of an A3RAg and an A2RAn for the manufacture of a pharmaceutical composition for inducing proliferation or differentiation of bone marrow or white blood cells.

37. Use of an active ingredient selected from the group consisting of an adenosine A3 receptor agonist (A3RAg), an adenosine A2 receptor antagonist (A2RAn) and a combination of an A3RAg and an A2RAn for the manufacture of a pharmaceutical composition for the prevention or treatment of leukopenia.

38. Use according to Claim 37, for the prevention or treatment of drug-induced leukopenia.

39. Use of an active ingredient selected from the group consisting of an adenosine A3 receptor agonist (A3RAg), an adenosine A2 receptor antagonist (A2RAn) and a combination of an A3RAg and an A2RAn for the manufacture of a

pharmaceutical composition for the prevention or treatment of toxic side effects of a drug.

40. Use according to Claim 39, wherein the toxic side effect is manifested by weight loss.

41. Use of an active ingredient from the group consisting of an adenosine A3 receptor agonist (A3RAg), an adenosine A2RAn and a combination of an A3RAg and an A2RAn for the manufacture of a pharmaceutical composition, wherein said manufacture comprises mixing said active ingredient with a drug that can cause a toxic side effect in a treated subject.

42. Use according to any one of Claims 38-41, wherein said drug is a chemotherapeutic drug.

43. Use according to any one of Claims 36-42, wherein the drug is formulated for oral administration.

44. A pharmaceutical composition for prevention or treatment of leukopenia, comprising an effective amount of an active ingredient selected from the group consisting of an adenosine A3 receptor agonist (A3RAg), an adenosine A2 receptor antagonist (A2RAn) and a combination of an A3RAg and an A2RAn and a pharmaceutically acceptable carrier.

45. A pharmaceutical composition according to Claim 44, for the prevention or treatment of drug-induced leukopenia.

46. A pharmaceutical composition for prevention or treatment of toxic side effects of a drug, comprising an effective amount of an active ingredient selected from the group consisting of an adenosine A3 receptor agonist (A3RAg), an adenosine A2 receptor antagonist (A2RAn) and a combination of two or three of these active ingredients and a pharmaceutically acceptable carrier.

47. A pharmaceutical composition according to Claim 46, wherein the toxic side effect is manifested by weight loss.

48. A pharmaceutical composition comprising, in combination a drug that can cause toxic side effect in a subject treated thereby and an active ingredient selected from the group consisting of an adenosine A3 receptor agonist (A3RAg), an

adenosine A2RAn and a combination of two or three of these active ingredients for the manufacture of a pharmaceutical composition for inducing proliferation or differentiation of bone marrow or white blood cells, said active ingredient being in an amount effective for prevention or treatment of the toxic side effects.

49. A pharmaceutical composition according to any one of Claims 45-48, wherein said drug is a chemotherapeutic drug.

50. A method for inhibiting abnormal cell growth in a subject, comprising administering to the subject a therapeutically effective amount of an active ingredient selected from the group consisting of an adenosine A3 receptor agonist (A3RAg), an adenosine A2 receptor agonist (A2RAg) and a combination of A3RAg and A2RAg.

51. A method according to Claim 50, for inhibiting growth or proliferation of tumor cells.

52. A method according to Claim 50, wherein the active ingredient is an A3RAg.

53. A method according to Claim 52, wherein the drug is administered orally.

54. A method according to Claim 50, wherein the drug is administered in combination with a chemotherapeutic drug.

55. A method according to Claim 50, wherein the active ingredient is that defined in any one of Claims 4-9.

56. Use of an active ingredient selected from the group consisting of an adenosine A3 receptor agonist (A3RAg), and adenosine A2 receptor agonist (A2RAg) and a combination of A3RAg and A2RAg for the manufacture of a pharmaceutical composition for inhibiting abnormal cell growth.

57. Use according to Claim 56, for the manufacture of a pharmaceutical composition for use in inhibiting growth or proliferation of tumor cells.

58. Use according to Claim 56 or 57, wherein said active ingredient is an A3RAg.

59. Use according to Claim 58, wherein the active ingredient is formulated for oral administration.

60. Use according to any one of Claims 56-59, wherein the pharmaceutical composition is intended for administration in combination with a chemotherapeutic drug.

61. Use according to any one of Claims 56-60, wherein the active ingredient is that defined in any one of Claims 4-9.

62. A pharmaceutical composition for inhibiting abnormal cell growth, comprising, as an active ingredient, a member of the group consisting of an adenosine A3 receptor agonist (A3RAg), an adenosine A2 receptor agonist (A2RAg) and a combination of A3RAg and A2RAg and a pharmaceutically acceptable carrier.

63. A pharmaceutical composition according to Claim 62, for inhibiting growth and proliferation of tumor cells.

64. A pharmaceutical composition according to Claim 62 or 63, wherein said active ingredient is an A3RAg.

65. A pharmaceutical composition according to Claim 64, being an oral composition and comprising a carrier acceptable for oral administration.

66. A pharmaceutical composition according to any one of Claims 62-65, for administration in combination with a chemotherapeutic drug.

67. A pharmaceutical composition according to any one of Claims 60-63, wherein the active ingredient is that defined in any one of Claims 4-9.

68. A method for treating cancer in a subject, comprising administering to the subject an effective amount of an adenosine A3 receptor agonist (A3Rag), the administration of the A3RAg yielding a dual effect in both inhibiting proliferation of cancer cells and countering toxic side effects of chemotherapeutic drug treatment of the same subject.

69. A method according to Claim 68, wherein the A3Rag synergizes with said drug to yield a stronger anti-tumor effect.

70. A method according to Claim 68, wherein the drug is administered orally.

71. A method according to Claim 68, wherein the active ingredient is that defined in any one of Claims 4-9.

72. Use of an A3Rag for the manufacture of a pharmaceutical composition for treatment of cancer in a subject to yield a dual effect in both inhibiting proliferation of cancer cells and countering toxic side effects of a chemotherapeutic drug treatment of the same subject.

73. Use according to Claim 72, wherein the active ingredient synergizes with said drug to yield a stronger anti-tumor effect.

74. Use according to Claim 72 or 73, wherein the active ingredient is formulated for oral administration.

75. Use according to any one of Claims 72-74, wherein the active ingredient is that defined in any one of Claims 4-9.

76. A pharmaceutical composition for use in treating cancer in a subject, comprising an amount of an adenosine A3 receptor agonist (A3RAg) effective to achieve a dual effect in inhibiting proliferation of tumor cells and countering toxic side effects of a chemotherapeutic drug treatment of the same subject.

77. A pharmaceutical composition according to Claim 76, wherein the A3Rag synergizes with said drug to yield a stronger anti-tumor effect.

78. A pharmaceutical composition according to Claim 76 or 77, being an oral composition.

79. A pharmaceutical composition according to any one of Claims 76-78, wherein the active ingredient is that defined in any one of Claims 4-9.

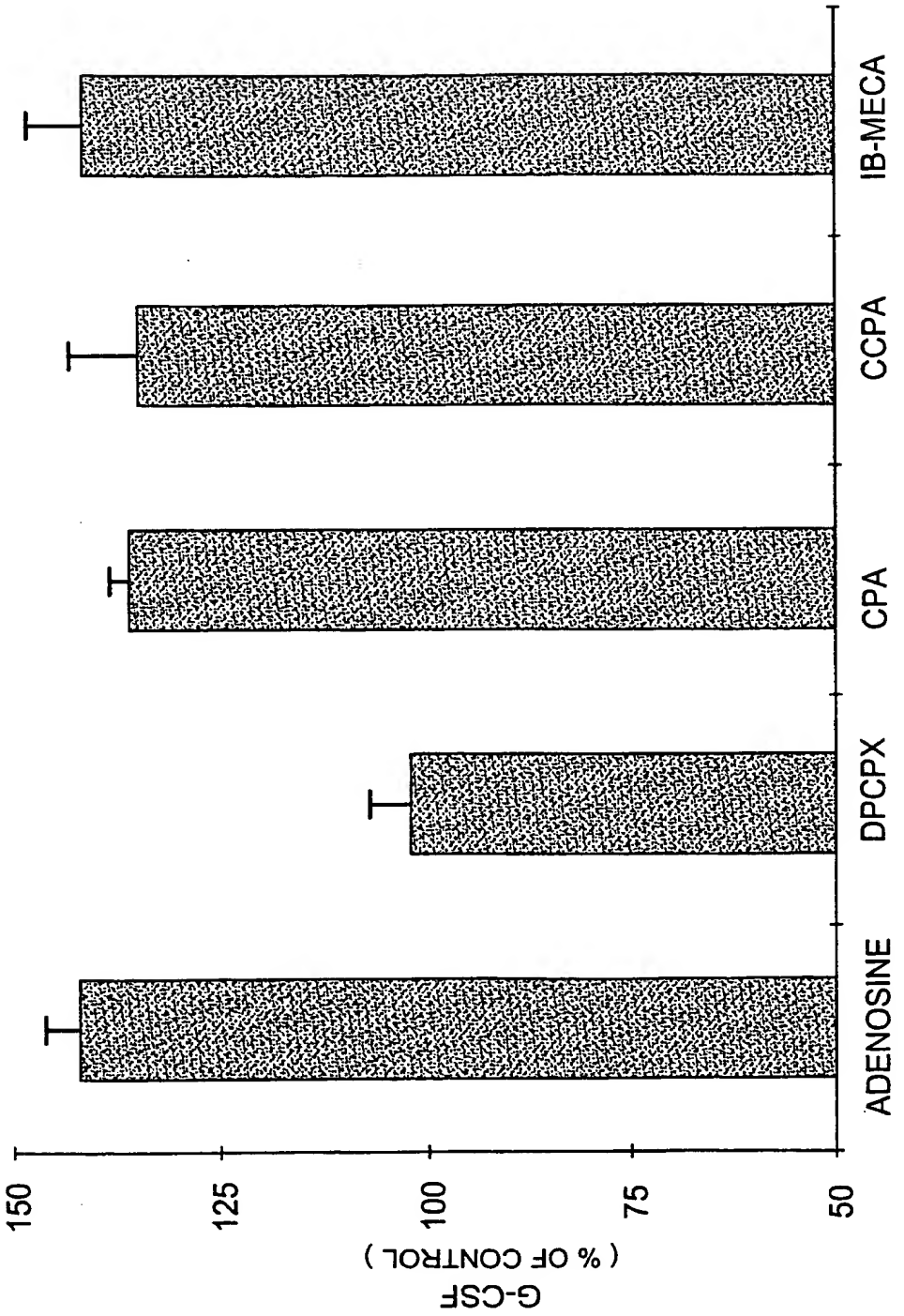

G-CSF
(% OF CONTROL)
150
125
100
75
50
ADENOSINE
DPCPX
CPA
CCPA
IB-MECA

FIG. 1

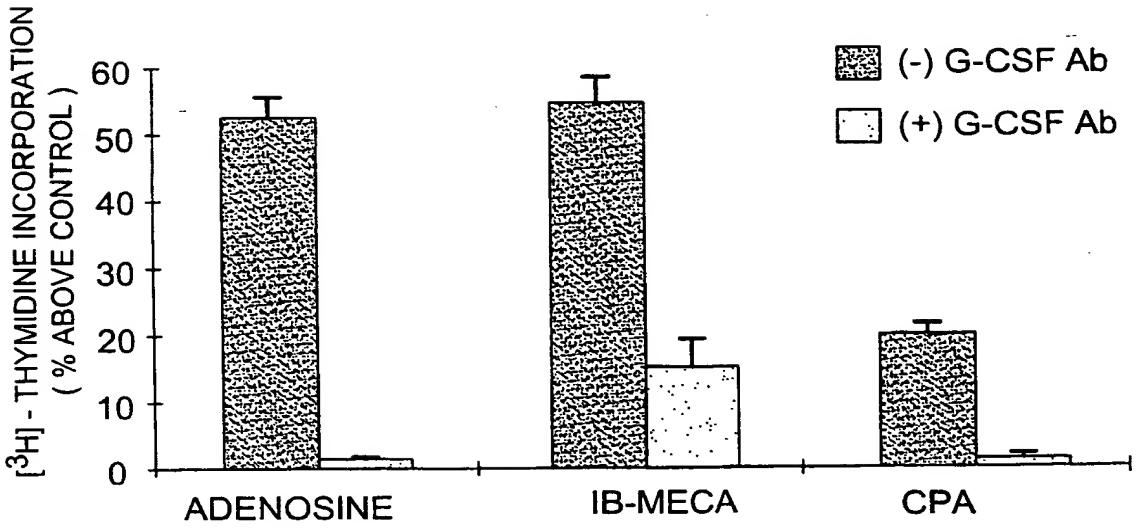

[3H] - THYMIDINE INCORPORATION
(% ABOVE CONTROL)
60
50
40
30
20
10
0
(-) G-CSF Ab
(+) G-CSF Ab
ADENOSINE
IB-MECA
CPA

FIG. 2

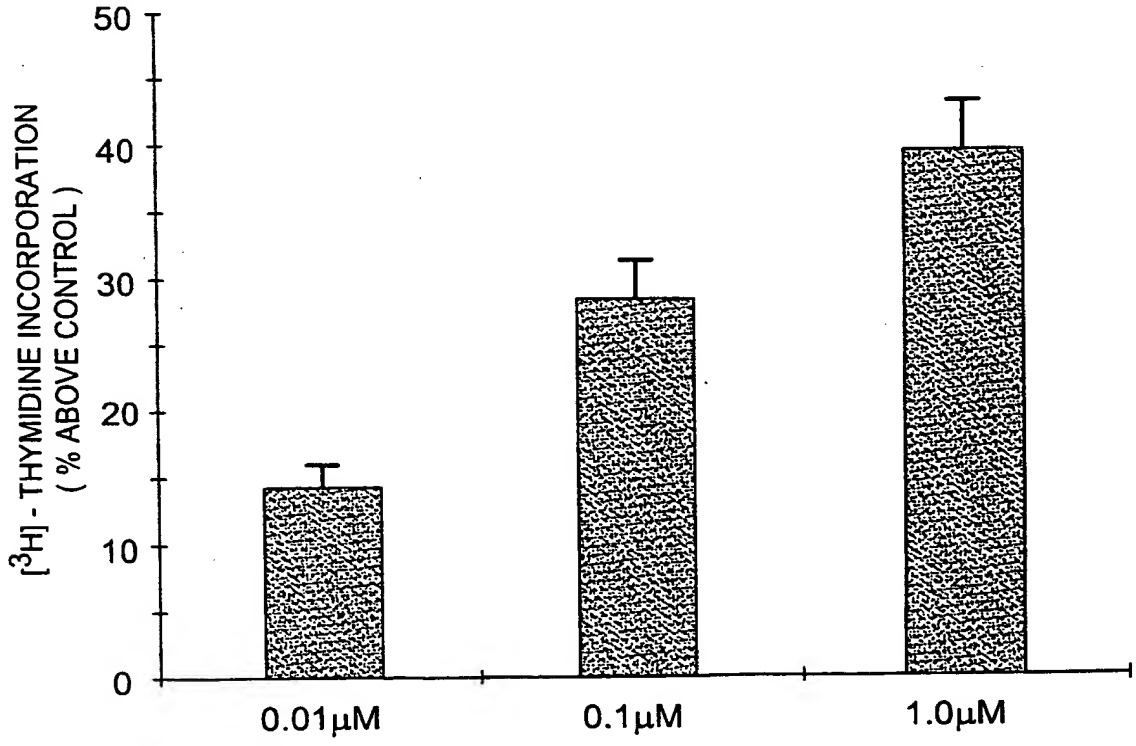

[3H] - THYMIDINE INCORPORATION
(% ABOVE CONTROL)
50
40
30
20
10
0
0.01μM
0.1μM
1.0μM

FIG. 4

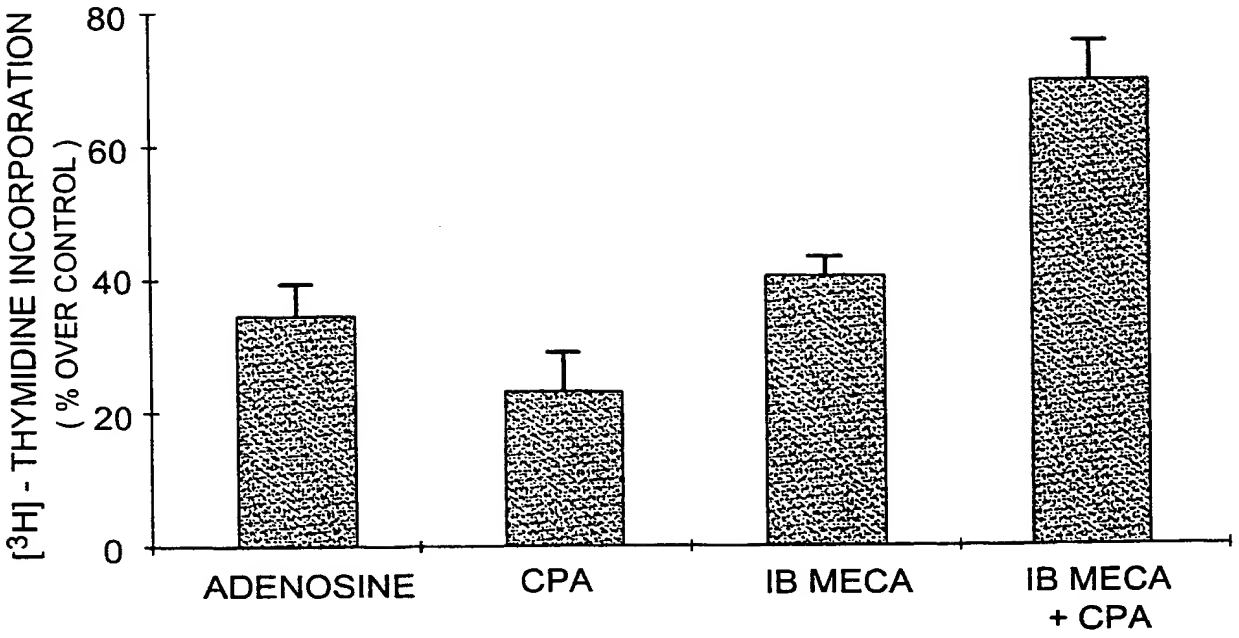

[3H] - THYMIDINE INCORPORATION
(% OVER CONTROL)
80
60
40
20
0
ADENOSINE
CPA
IB MECA
IB MECA
+ CPA

FIG. 3A

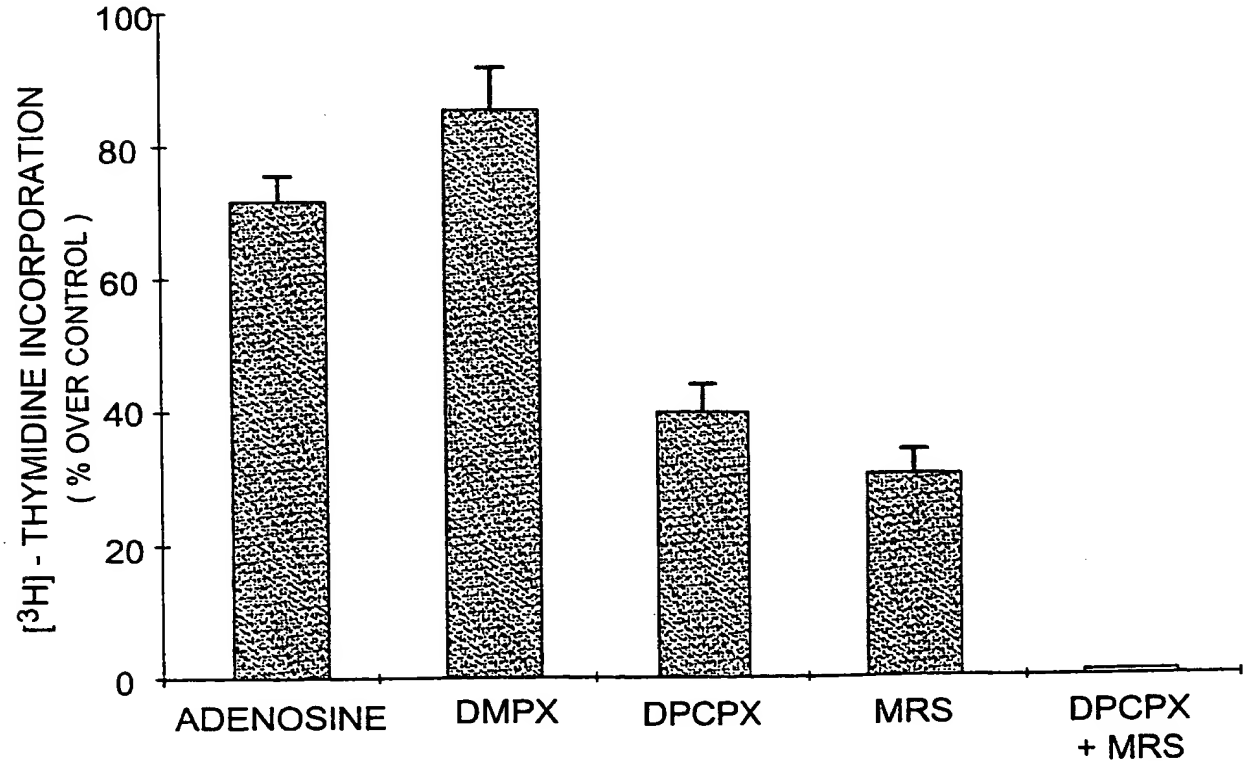

[3H] - THYMIDINE INCORPORATION
(% OVER CONTROL)
100
80
60
40
20
0
ADENOSINE
DMPX
DPCPX
MRS
DPCPX
+ MRS

FIG. 3B

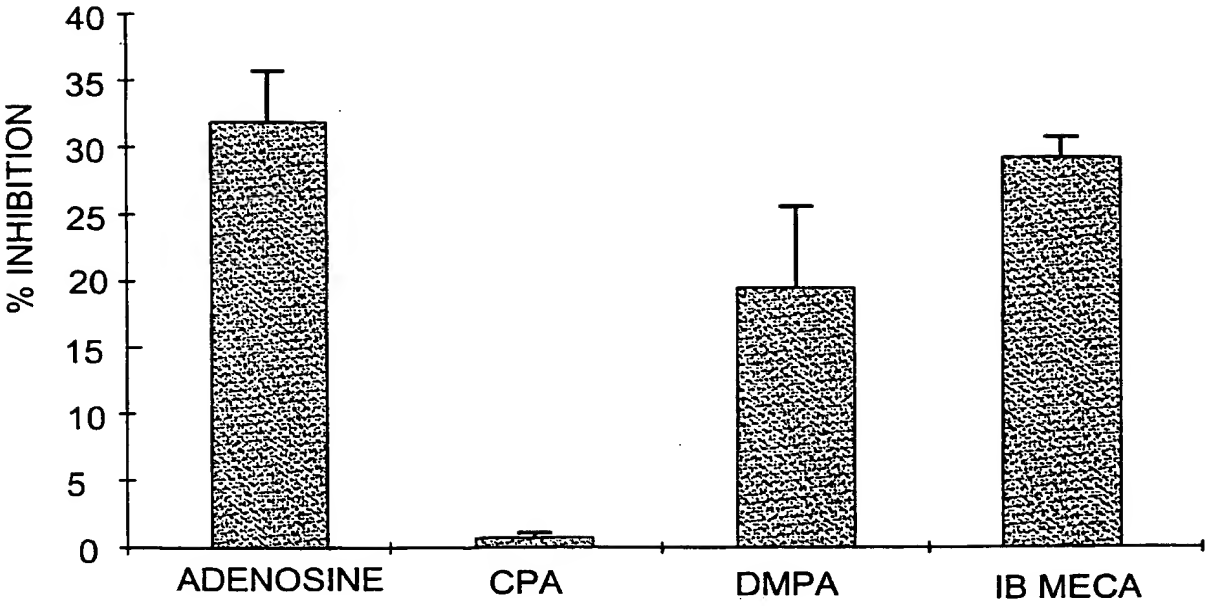

% INHIBITION
40
35
30
25
20
15
10
5
0
ADENOSINE
CPA
DMPA
IB MECA

FIG. 5A

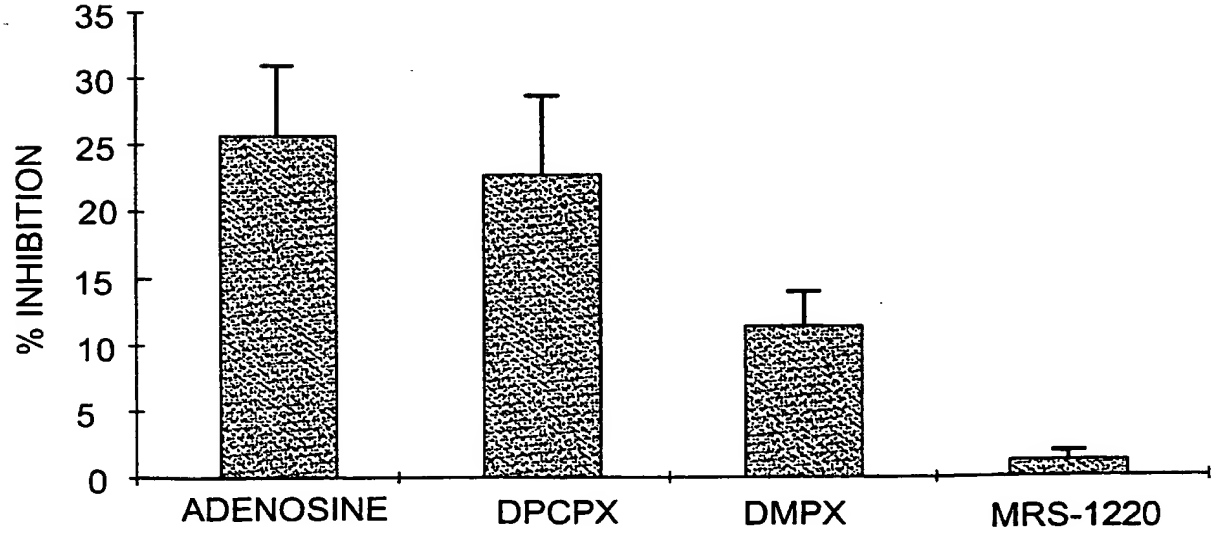

% INHIBITION
35
30
25
20
15
10
5
0
ADENOSINE
DPCPX
DMPX
MRS-1220

FIG. 5B

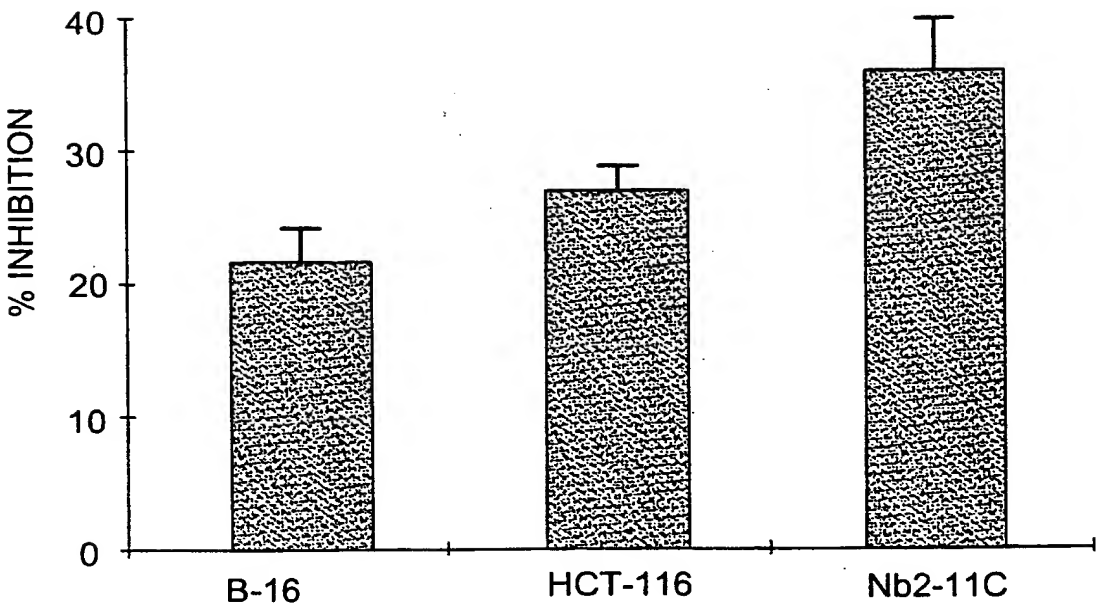


FIG. 6

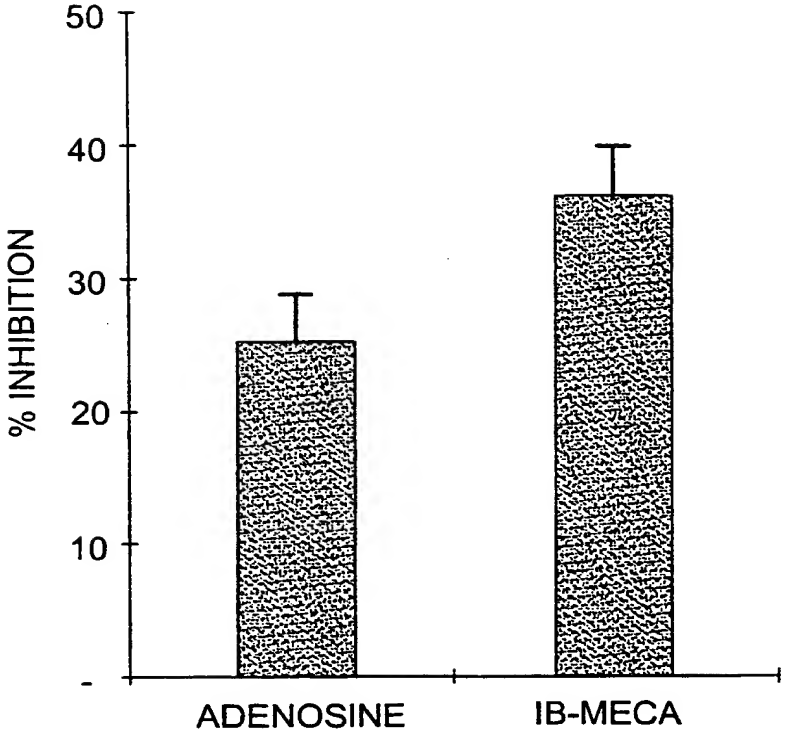

50
40
30
20
10
-
% INHIBITION
ADENOSINE
IB-MECA

FIG. 7A

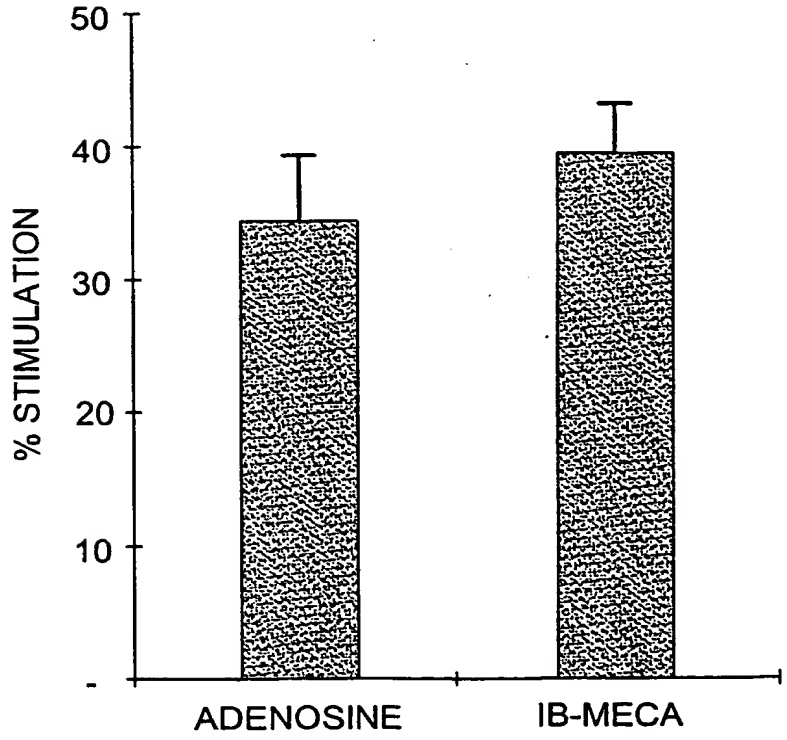

50
40
30
20
10
-
% STIMULATION
ADENOSINE
IB-MECA

FIG. 7B

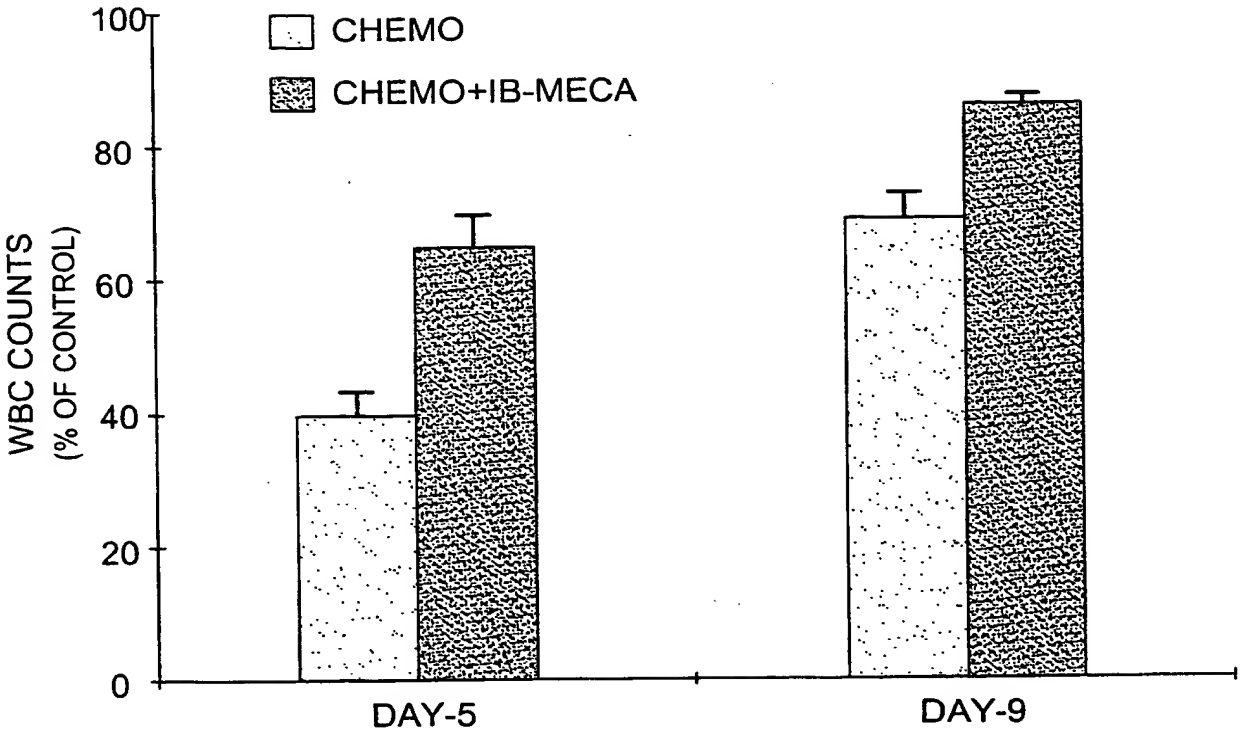

CHEMO
CHEMO+IB-MECA
WBC COUNTS
(% OF CONTROL)
100
80
60
40
20
0
DAY-5
DAY-9

FIG. 8

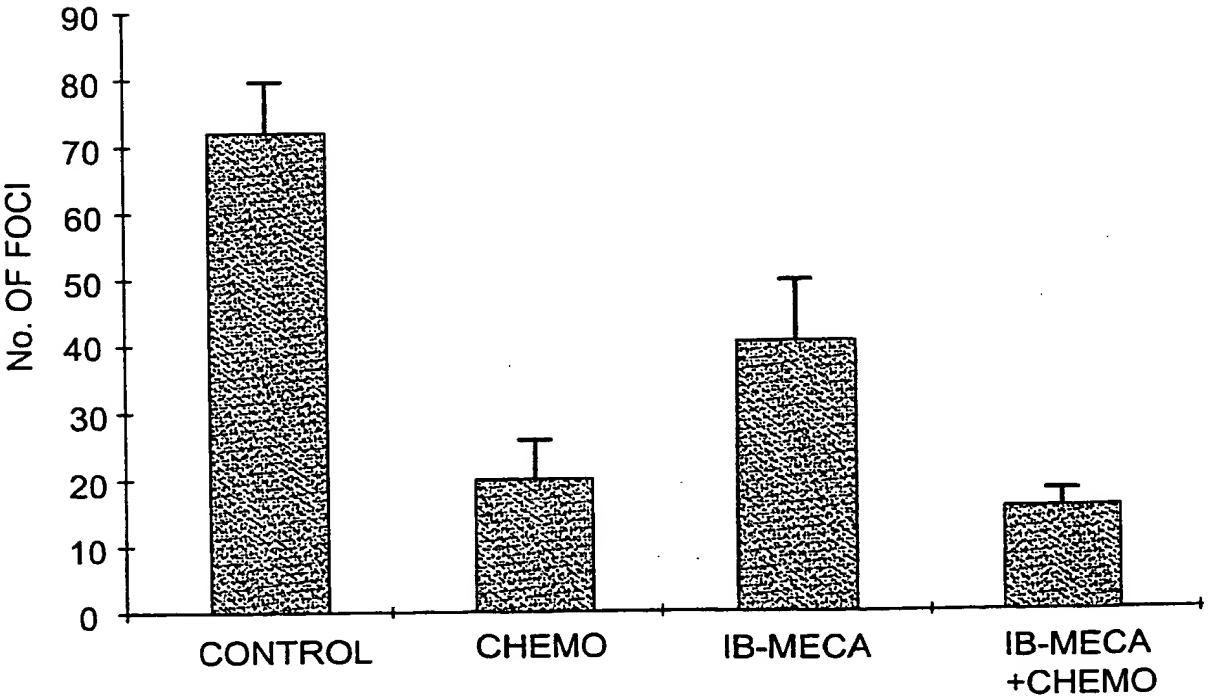

No. OF FOCI
90
80
70
60
50
40
30
20
10
0
CONTROL
CHEMO
IB-MECA
IB-MECA
+CHEMO

FIG. 9

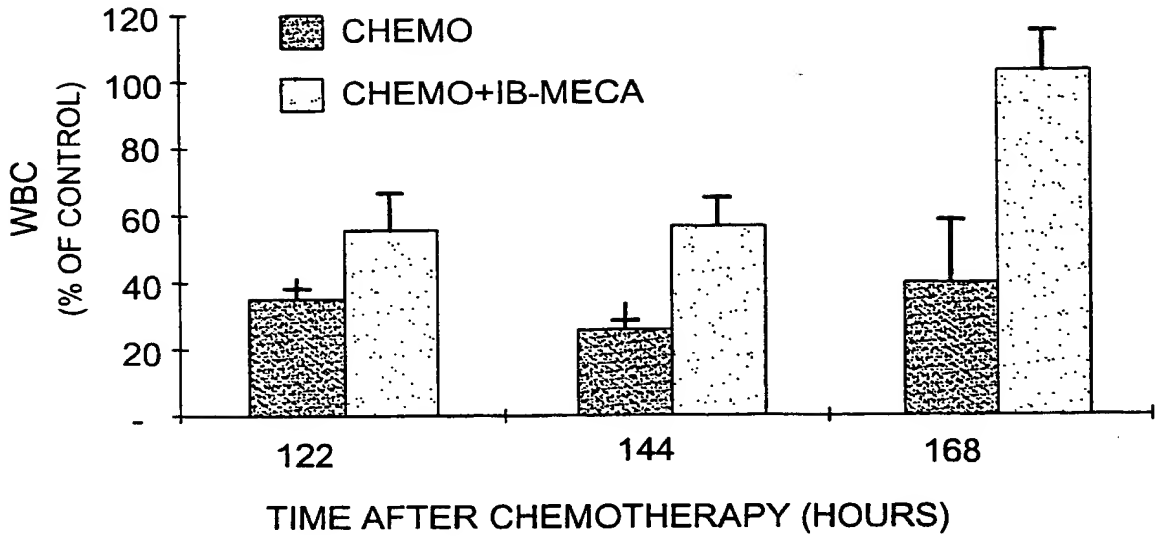


FIG. 10A

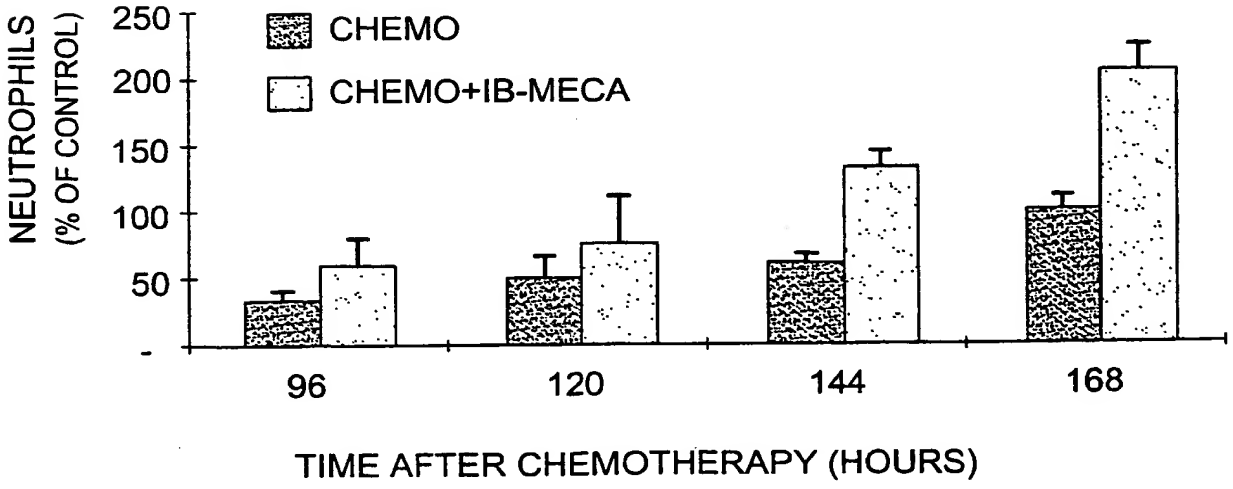


FIG. 10B

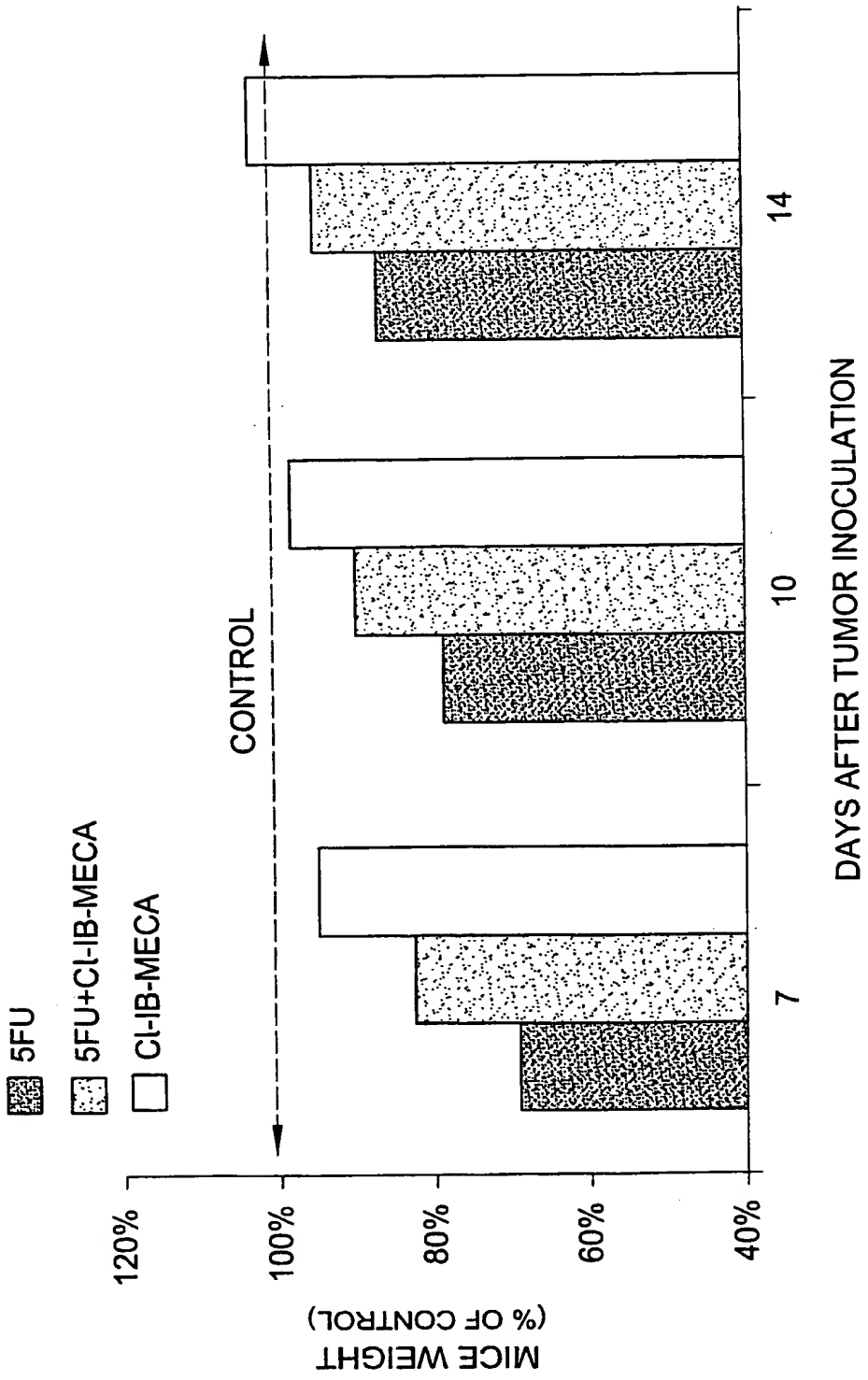

5FU
5FU+Cl-IB-MECA
Cl-IB-MECA
CONTROL
120%
100%
80%
60%
40%
MICE WEIGHT
(% OF CONTROL)
7
10
14
DAYS AFTER TUMOR INOCULATION

FIG. 11

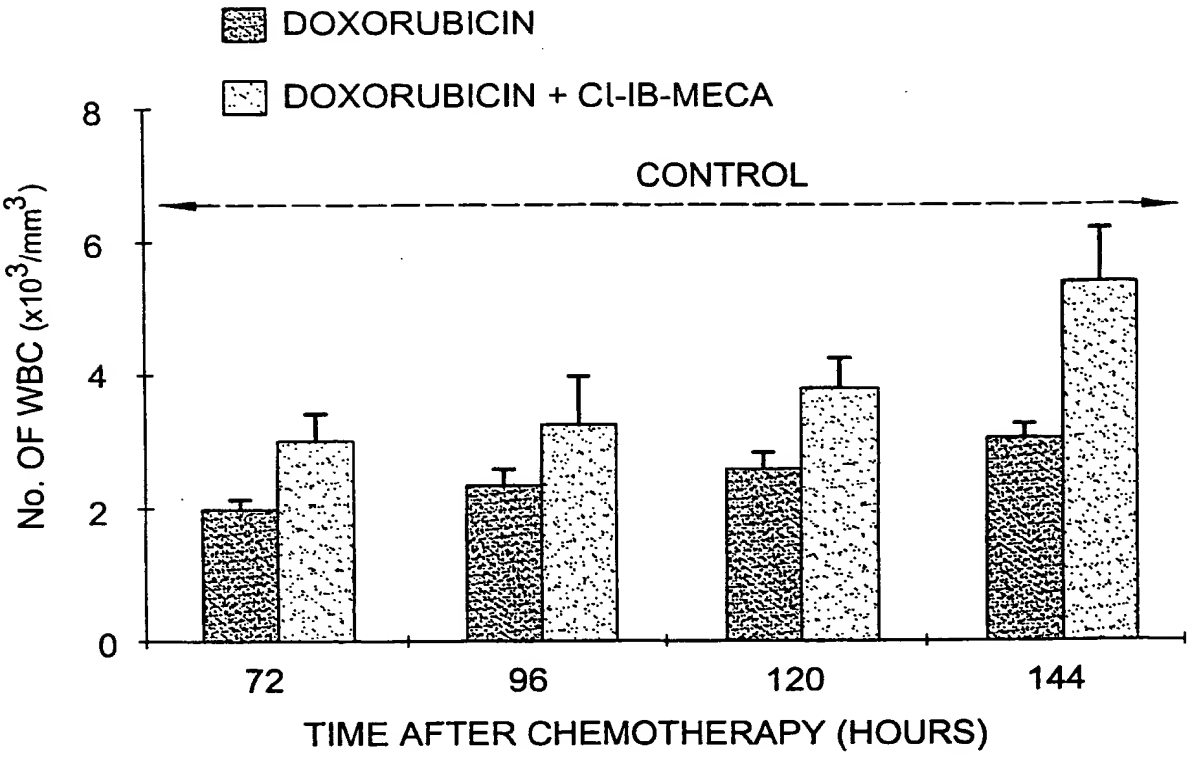


FIG. 12A

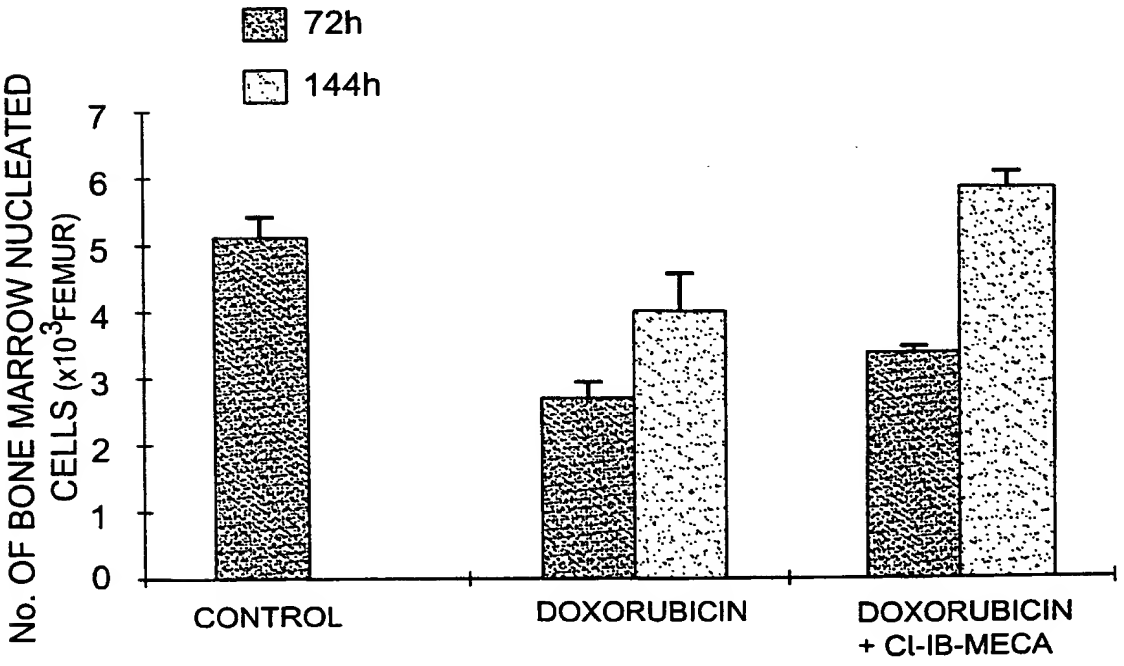


FIG. 12B

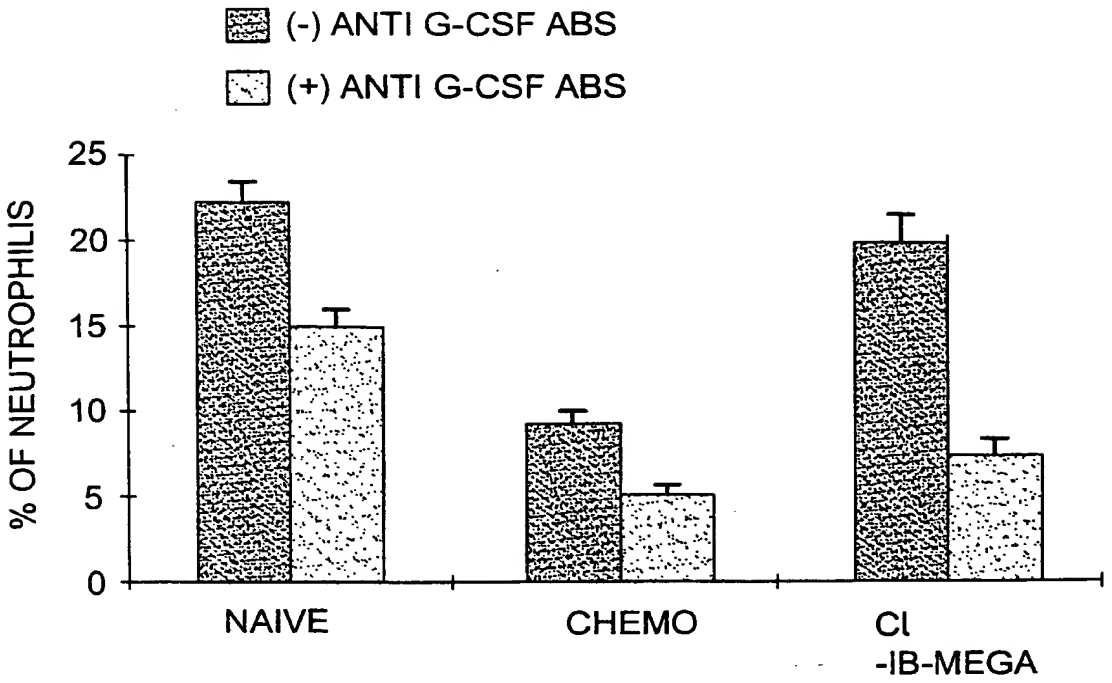

(-) ANTI G-CSF ABS
(+) ANTI G-CSF ABS
% OF NEUTROPHILIS
25
20
15
10
5
0
NAIVE
CHEMO
Cl
-IB-MEGA

FIG. 13

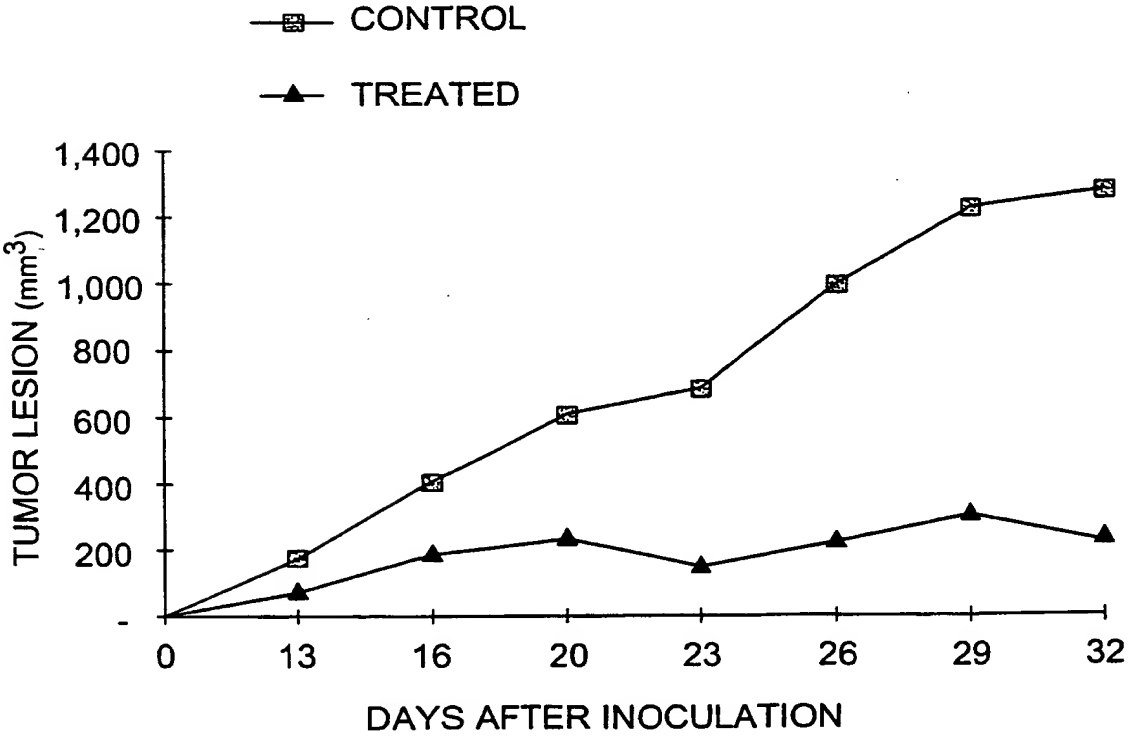

CONTROL
TREATED
TUMOR LESION (mm3)
1,400
1,200
1,000
800
600
400
200
-
0
13
16
20
23
26
29
32
DAYS AFTER INOCULATION

FIG. 14

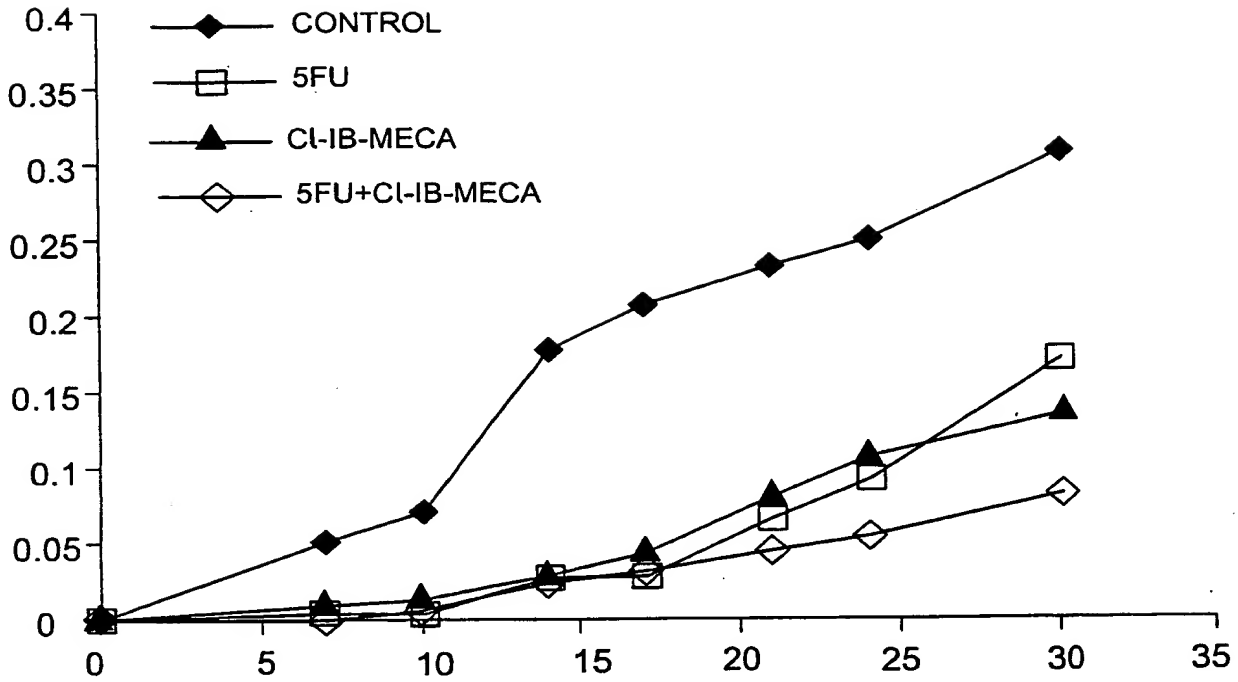

CONTROL
5FU
Cl-IB-MECA
5FU+Cl-IB-MECA
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
5
10
15
20
25
30
35

FIG. 15

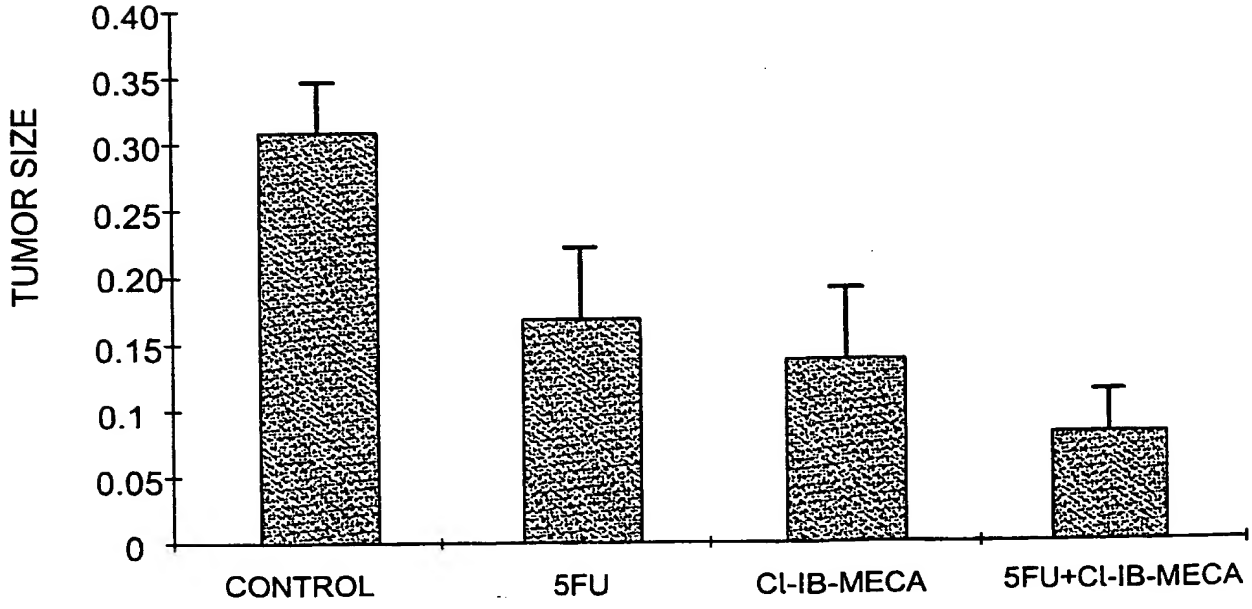

TUMOR SIZE
0.40
0.35
0.30
0.25
0.20
0.15
0.1
0.05
0
CONTROL
5FU
Cl-IB-MECA
5FU+Cl-IB-MECA

FIG. 16

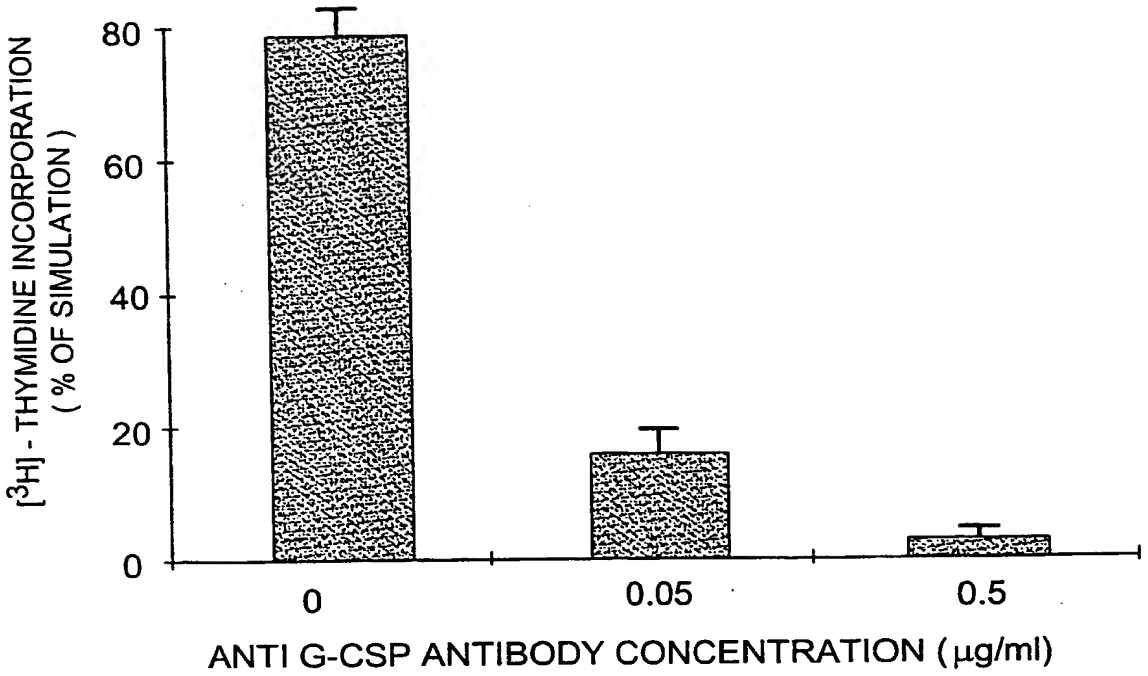

80
60
40
20
0
[3H] - THYMIDINE INCORPORATION
(% OF SIMULATION)
0
0.05
0.5
ANTI G-CSP ANTIBODY CONCENTRATION (μg/ml)

FIG. 17

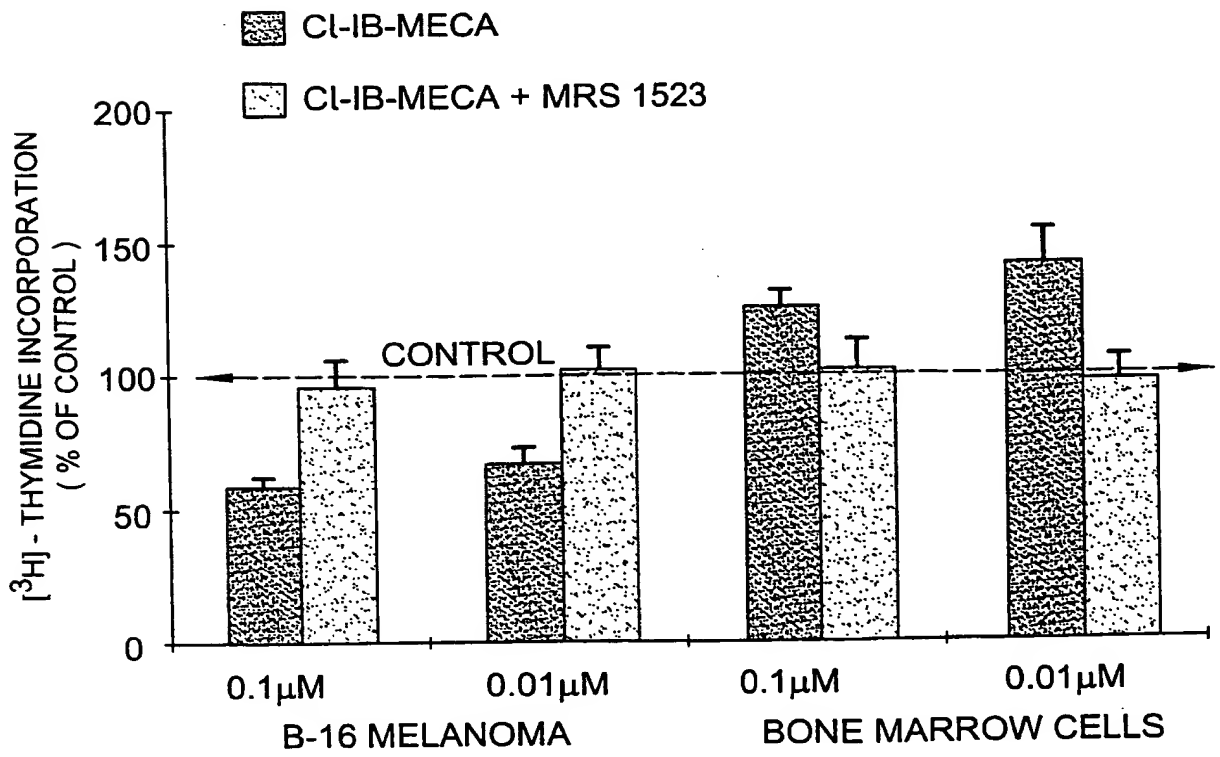

Cl-IB-MECA
Cl-IB-MECA + MRS 1523
200
150
100
50
0
[3H] - THYMIDINE INCORPORATION
(% OF CONTROL)
CONTROL
0.1μM
0.01μM
0.1μM
0.01μM
B-16 MELANOMA
BONE MARROW CELLS

FIG. 18

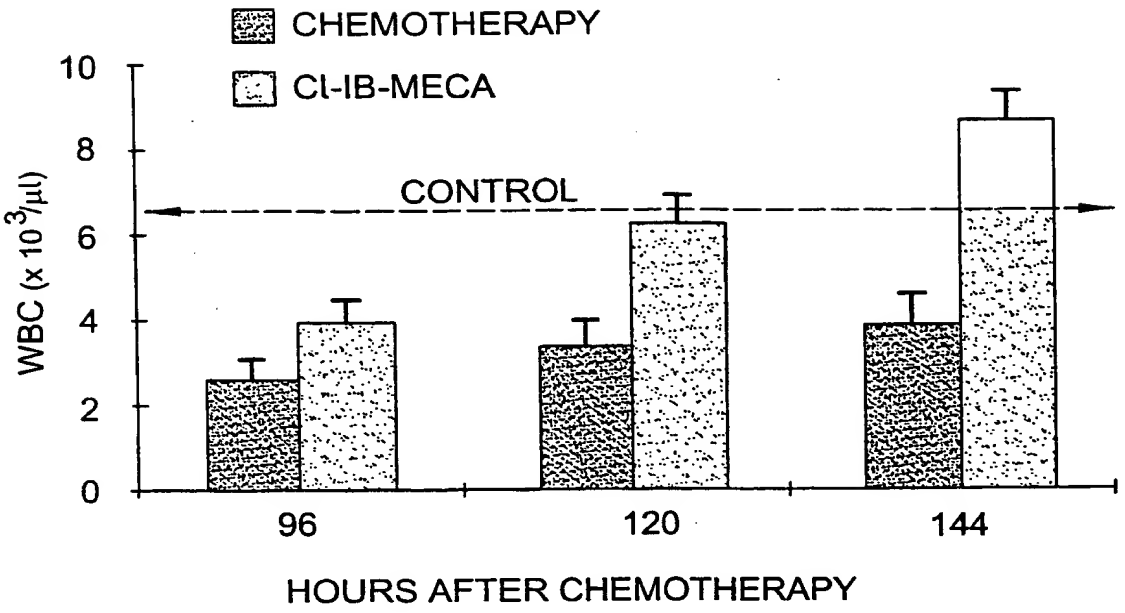


FIG. 19A

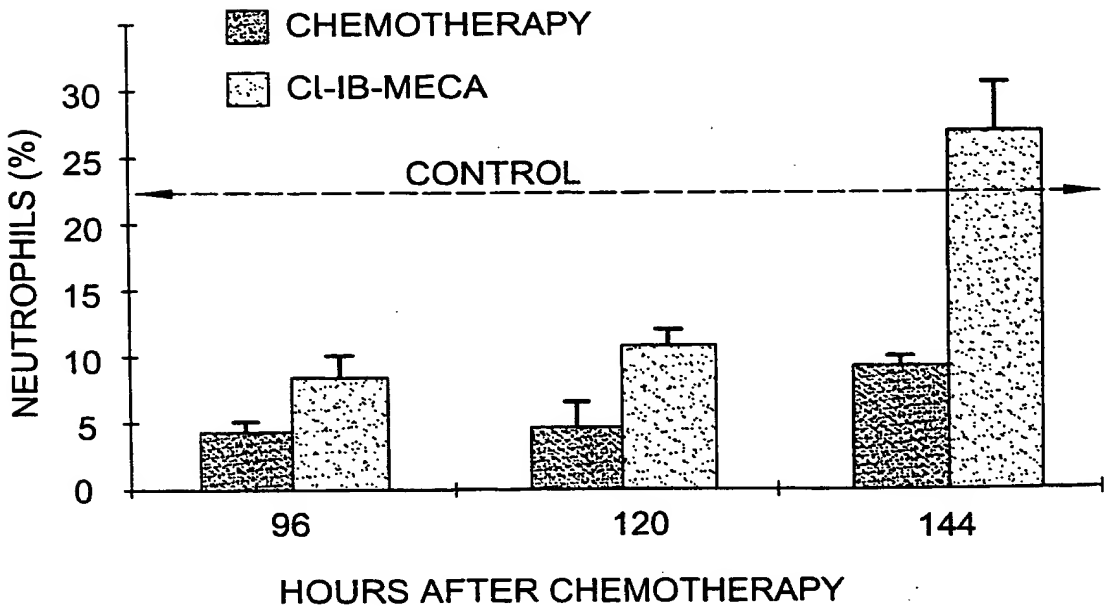


FIG. 19B

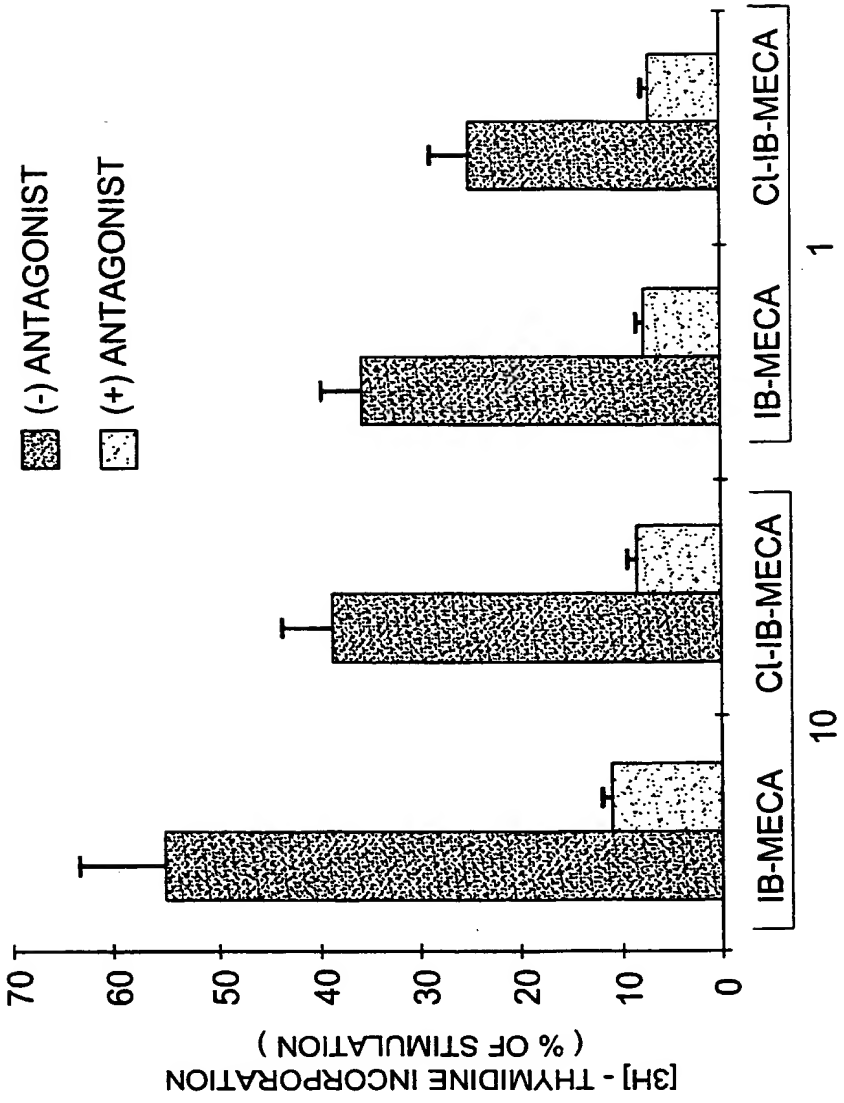

[3H] - THYMIDINE INCORPORATION
(% OF STIMULATION)
70
60
50
40
30
20
10
0
IB-MECA
Cl-IB-MECA
10
IB-MECA
Cl-IB-MECA
1
(-) ANTAGONIST
(+) ANTAGONIST

FIG. 20

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 March 2001 (22.03.2001)

PCT

(10) International Publication Number
WO 01/019360 A3

(51) International Patent Classification[7]: A61K 31/00, 31/7052, 31/7076, 31/708, 31/706, A61P 39/00, 35/00

(21) International Application Number: PCT/IL00/00550

(22) International Filing Date:
8 September 2000 (08.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

131864	10 September 1999 (10.09.1999)	IL
133680	23 December 1999 (23.12.1999)	IL

(71) Applicant *(for all designated States except US)*: **CAN-FITE BIOPHARMA LTD.** [IL/IL]; 10 Bareket Street, Petach Tikva 49170 (IL).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **FISHMAN, Pnina** [IL/IL]; Asher Barash Street 19, 46365 Herziliya (IL).

(74) Agent: **REINHOLD COHN AND PARTNERS**; P.O. Box 4060, 61040 Tel Aviv (IL).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
19 September 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/019360 A3

(54) Title: PHARMACEUTICAL COMPOSITIONS COMPRISING AN ADENOSINE RECEPTOR AGONIST OR ANTAGONIST

(57) Abstract: Adenosine receptor agonists, particularly an agonist which binds to the A3 adenosine receptor, are used for induction of production or secretion of G-CSF within the body, prevention or treatment of toxic side effects of a drug or prevention or treatment of leukopenia, particularly drug-induced leukopenias; and inhibition of abnormal cell growth and proliferation.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 00/00550

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/00 A61K31/7052 A61K31/7076 A61K31/708 A61K31/706
A61P39/00 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, BIOSIS, EPO-Internal, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 50047 A (TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA) 12 November 1998 (1998-11-12) the whole document page 11, line 12 - line 35 page 12, line 15 - line 35	20-22, 29,39,46
A	---	1-19
X	WO 94 21195 A (GENSIA INC.) 29 September 1994 (1994-09-29) see the whole document, especially page 6 lines 20-25	20-22
A	---	1-8
A	WO 95 02604 A (THE UNITED STATES OF AMERICA) 26 January 1995 (1995-01-26) cited in the application see the whole document, especially page 36	1-28
	--- -/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 June 2002	14 06. 2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gac, G

Form PCT/ISA/210 (second sheet) (July 1992)

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A	US 5 688 774 A (KENNETH A.J.) 18 November 1997 (1997-11-18) the whole document	1-8
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 63938 A (HILL JEFFREY L ;NYCE JONATHAN W (US); EPIGENESIS PHARMACEUTICALS I) 16 December 1999 (1999-12-16) page 9 lines 10,14,19,22,31-39 page 11, paragraph 1 page 12, last paragraph page 13, paragraph 1 page 38, paragraphs 4,5 page 45, paragraph 7 page 50 page 60; example 28 example 39 figure 7	20,22, 29,31, 39, 41-43, 46,47, 50-54, 56-60, 62-66, 76-78
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL 00/00550

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 23-35,50-55,68-71 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [X] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

1-22,29-31,39-43,46,47,50-79 (subjects 2, 3 and 6) and subject 1 : thus claims 1-79 (all partially)

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

This supplemental sheet is intended to raise objections based on the total of inventions for which (additional) fees have been paid after the notice of lack of unity of invention: i.e. it concerns inventions 1, 2, 3 and 6.

* The expressions "adenosine A3 receptor agonist", "adenosine A1 receptor agonist", "adenosine A2 receptor antagonist", "adenosine A2 receptor agonist", "a drug", "a chemotherapeutic drug" relate to compounds which are actually not well-defined and may encompass an extremely large and undefined number of different compounds. Moreover, formulas of claims 4-6 and 9 relate to an extremely large number of possible structures. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed.
* The expressions " (achieving a therapeutic effect comprising) inducing G-CSF secretion or production", "inducing proliferation or differentiation of bone marrow or white blood cells", "inhibiting abnormal cell growth" are not well-defined therapeutical applications for the compounds claimed herein.
* Under the general cover of "toxic side effects of a drug", a great and unlimited number of symptoms, disorders or diseases as well as drugs can be included and it is not clear which ones are meant herein. Moreover, only one symptom (weight loss) and two drugs (cyclophosphamide and 5-fluorouracile) are sufficiently well-defined and supported by the description to allow a meaningful search to be performed (Article 6 PCT). The same objections apply to the synergetic use of combinations with "chemotherapeutic drug" for cancer therapy (only doxorubicin combinations are sufficiently supported by the description).

In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Since both the compounds and the therapeutical applications are not well-defined (as mentioned above), the claims refering to said expressions or formulas are considered to lack clarity in the sense of Article 6 PCT to such an extend as to render a complete meaningful search impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be clear, concise and supported , namely those parts concerning:

* the A3 agonists of claims 7 and 8 only in relation to the treatment of (drug-induced) myelotoxicity , (drug-induced) leukopenia (and neutropenia, blood levels of circulating leukocytes) as well as on the underlying general inventive concept (G-CSF stimulation).

* these A3 agonists in relation to cancer treatment (with or without dual effect) and (also independently) to the mixtures or interactions with 5-Fluorouracile, cyclophosphamide or doxorubicin.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

* the A3 agonists of claims 7 and 8 in relation to the treatment of drug-induced weight loss.

* the A1 agonists: CPA and CCPA mentioned on page 26 and pages 31-32 of the present description, in relation to their activity on (drug-induced) myelotoxicity, (drug-induced) leukopenia (and neutropenia, blood levels of circulating leukocytes) , as well as on the underlying general inventive concept (G-CSF stimulation).

* the A2 antagonist DPMX in combination with A3 agonists of claims 7 and 8, independently or in relation to drug-induced weight loss, as well as on the underlying general inventive concept.

* the A2 agonist DPMA in combination/interaction/synergy with the A3 agonists of claims 7 and 8, independently or in relation to cancer, as well as on the underlying general inventive concept.

CONCLUSION :
++++++++++

Concerning invention number 1:
claims searched partially (incompletely): 1-28,32-38,44,45,48,49.

Concerning inventions numbers 2, 3 and 6:
claims searched partially (incompletely): 1,4,9-10,16,20,22, 29,31,39,41-43,46-47,50-54,56-79 .

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-28,32-38,44-45,48,49 (all partially)

   Use of (and pharmaceutical compositions containing) A3 adenosine receptor agonists to treat drug-induced myelotoxicity, to induce proliferation or differentiation of bone marrow or white blood cells or to prevent or treat (drug-induced) leukopenia (and neutropenia), for elevating blood levels of circulating leukocytes, possibly in combination with A1 adenosine agonists or A2 adenosine antagonists or with a drug that can cause toxic side effects (in relation to these uses).

2. Claims: 1-22 (all partially)

   Use of (and pharmaceutical compositions containing) A1 adenosine receptor agonists to treat drug-induced myelotoxicity, to induce proliferation or differentiation of bone marrow or white blood cells or to prevent or treat (drug-induced) leukopenia (and neutropenia), for elevating blood levels of circulating leukocytes, as far as not already covered by previous subject.

3. Claims: 29-31,39-43,46,47 (all partially)

   Use of (and pharmaceutical compositions containing) an A3 adenosine receptor agonist, possibly in combination with an A2 adenosine receptor antagonist or with a drug that can cause toxic side effects, to treat toxic side effects of a drug (weight loss).

4. Claims: 50-51,54,56,57,59,60,62,63,66 (all partially)

   Use of an A2 adenosine receptor agonist, alone or in combination with a chemotherapeutic/anti-tumor drug, to inhibit abnormal cell growth and compositions thereof for this use, as far as not already covered by previous inventions.

5. Claims: 23,27-28,32,36-38,48-49

   Use of (and pharmaceutical compositions containing) an A2 adenosine receptor antagonist, possibly in combination with a drug that can cause toxic side effects, to induce proliferation or differentiation of bone marrow or white blood cells or to prevent or treat (drug-induced) leukopenia (and neutropenia), for elevating blood levels of circulating leukocytes, as far as not already covered by previous invention.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

6. Claims: 50-79 (all partially)

Use of an A3 adenosine receptor agonist, alone or in combination with an A2 adenosine receptor agonist or with a chemotherapeutic/anti-tumor (synergetic) drug, to inhibit abnormal cell growth, in particular tumor cell growth and to treat cancer, wherein said A3R agonist may have a dual effect of both inhibiting proliferation of cancer cells and counteracting toxic side effects of a chemotherapeutic drug (and compositions thereof), as far as not already covered by previous inventions.

7. Claims: 50-51,54,56,57,59,60,62,63,66 (all partially)

Use of an A2 adenosine receptor agonist, alone or in combination with a chemotherapeutic/anti-tumor drug, to inhibit abnormal cell growth and compositions thereof for this use, as far as not already covered by previous inventions.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IL 00/00550

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PCT/IL 00/00550

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